CHARACTERIZATION OF HUMAN ROTA VIRUS GROUP A SEROTYPES CAUSING GASTROENTERITIS AMONG CHILDREN BELOW FIVE YEARS AND HIV-INFECTED ADULTS OF VIWANDANI SLUM IN NAIROBI, KENYA

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Characterization of human rota virus group a serotypes causing gastroenteritis among children below five years and hiv-infected adults of viwandani slum in Nairobi, Kenya

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

Sign…….. Date……

Sandra Kendra Raini

This thesis has been submitted for examination with our approval as University Supervisors.

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DEDICATION

I dedicate this work to my parents, Mr. Samwel Raini and Mrs. Elizabeth Raini, and my beloved daughter Mary Leigh Okoth. Your efforts and belief in my ability have been a daily source of inspiration and motivation towards completion of this work.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABBREVIATIONS AND ACRONYMS

AIDS	Acquired Immune-deficiency Syndrome
ART	Antiretroviral Therapy
CCC	Comprehensive Care Centre
cDNA	Complementary DNA
CVR	Centre for Virus Research
DNA	Deoxyribonucleic Acid
ELISA	Enzyme-linked Immunosorbent Assay
ERC	Ethical Review Committee
GE	Gastroenteritis
HIV	Human immunodeficiency virus
IgG	Immunoglobulin G
ITROMID	Institute of Tropical Medicine and Infectious diseases.
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KEMRI	Kenya Medical Research Institute
ORT	Oral Rehydration Therapy.
PCR	Polymerase chain reaction
SSC	Scientific Steering Committee

ABSTRACT

Rotavirus remains a leading cause of severe diarrhoea in children worldwide, especially in developing countries where about 2000 children die each day from rotavirus-related gastroenteritis infection. Due to HIV/AIDS scourge in Kenya, it is possible that rotavirus caused gastroenteritis has been aggravated. The Global Alliance for Immunizations has ranked rotavirus as a priority for vaccine. To ensure the success of this, it is important to document the local strain(s) of rotavirus in circulation in various regions. This study was aimed at characterizing human rotavirus group A serotypes causing gastroenteritis among children below 5 years of age and HIV-infected adults in Viwandani slum, Nairobi. A cross-sectional, hospital based study was conducted and a total of 260 faecal specimen samples were collected for analysis (128 from children and 132 from HIV infected adults) during the period between August 2012- July 2013 from two health centres in viwandani slums in Nairobi. The samples were detected for rotavirus strains using antigen based enzyme immune-sorbent assay (ELISA) to detect the prevalence of rotavirus infection, Polyacrylamide gel electrophoresis was used to detect rotavirus electropherotypes and finally genotyping was done by RT-PCR to confirm rotavirus genotypes using genotype-specific primer sets targeting VP4 and VP7 genes. Rotavirus was detected in 23% and 8% of children and adults respectively. This study found out that rotavirus was in circulation throughout the year however**,** with high incidence cases being detected during wet and cold months of March April and November and dry month of August. Prevalence was high among children aged two yrs and adults above age of 48 yrs. The common globally distributed strains, G1 and G3, accounted for 60% detections while the unusual G9 strain accounted for 80% infection in adults. G1[P8] was the common genotypic combination in children, accounting for 40% infection, whereas G9[P8] accounted for 60% of the infection in adults. This study concludes that there is strain diversity in rotavirus circulating in Viwandani slums in Nairobi. In addition, the study asserts that the two rotavirus vaccines recommended for world, cover all the circulating in Viwandani. It is recommended that molecular epidemiology of rotavirus especially in low income settlement be a continuous process especially among HIV infected.

CHAPTER ONE
INTRODUCTION

1.1 Background Information

Severe diarrhoea due to rotavirus infection remains a major public health concern in both developed and developing countries (Temu *et al*, 2011). It leads to high rates of morbidity and mortality globally (Kang *et al*, 2005). Rotavirus is the single most important etiological agent implicated in severe diarrhoea in children and is subject of intense vaccine research (Carraro *et al*, 2008). Virtually every child contracts an episode of diarrhoea in the first five yrs of their life (Basu *et al*, 2003). It is estimated that 2.4-3.3 million deaths occur annually in developing countries due to rotavirus caused gastroenteritis (Kiulia *et al*, 2014). Further, a study covering 10 countries in Sub-Saharan Africa observed that >40% of all episodes of acute gastroenteritis affecting children under 5 years occur due to rotavirus infection (Mwenda *et al*, 2010). The burden of the disease has also been experienced in the health sector where >40% of hospital admissions of acute gastroenteritis infections in children under 5 years are due to rotavirus infections (Mwenda *et al*, 2010). In 2008, the WHO reported rotavirus to be the most common cause of severe diarrheal disease hospitalizations in young children in Africa. In fact some studies carried out in Kenya have shown a high prevalence of 29% of rotavirus infections among hospitalized patients (Nokes *et al*, 2008) as compared to only 18% of outpatient cases (Kiulia *et al*, 2006). These findings of high burden of rotavirus in hospitalized children not only puts pressure on the limited bed capacity in many African nations, but also on the economy, as women who contribute significantly to the economies spend a lot of time in hospitals with their children away from work. Further a lot of time is also wasted by children who otherwise would spend their time in schools.

Gastroenteritis is an inflammation of the gastrointestinal tract involving both the stomach and small intestine resulting in acute diarrhoea and vomiting Grimes *et al*, 2002). The aetiologies of gastroenteritis include certain viruses, bacteria, their toxins, parasites, or an adverse allergic reaction to foods or medication (Cárcamo *et al*, 2005). Rotavirus infection is mostly spread via oral-faecal route although respiratory transmission could also occur. Improvement in water, personal hygiene, living

standards and sanitation are inadequate measures in prevention of rotavirus diarrhoea. These measures could reduce transmission of rotavirus but they are unlikely to reduce incidence of rotavirus diarrhoea significantly hence, use of vaccines (Steele & Glass, 2011a).

The main symptoms of rotavirus gastroenteritis are fever, abdominal pain, lethargy, diarrhoea and vomiting that may lead to hypovolemic shock and dehydration (Elhag *et al,* 2013)**.** Severe cases may be fatal and the World Health Organization (WHO) estimates that 527,000 children under the age of five years die of rotavirus disease each year and almost half of these deaths (230,000) occur in Sub-Saharan Africa (Parashar *et al,* 2006)**.** There are also the most important cause of early childhood non-bacterial gastroenteritis with illnesses also being observed in older children and adults (Marrie *et al,* 1982) .

Contrary to the belief of rotavirus infection occurring only in children, studies have reported rotavirus infection in adults (Al-Thani *et al,* 2013). There is limited data documented on the genotypic diversity of rotavirus and various strains in adults that may reduce the effectiveness of rotavirus vaccine program (Shen *et al,* 2013). Rotavirus infections of adults are usually subclinical but occasionally cause illness in parents of children with rotavirus diarrhoea, immune-compromised patients (including those with HIV), the elderly, and travellers to developing countries (Marrie *et al,* 1982). Rotavirus infection in subjects with insufficient immunity may be severe (Anderson and Weber, 2004). These infections in adults can be asymptomatic while in other occasions they can manifest in a wide variety of symptoms.

Transmission from children to adults has come out significantly in a majority of earlier studies Anderson & Weber (2004). Endemic disease, epidemic outbreaks and travel related infections are other most important epidemiological setting for rotavirus infection in adults. With the high level of poverty and the effects of HIV/AIDS scourge in many Sub-Saharan African countries, an increased burden of rotavirus infection would be expected among the immunocompromised populations who take care of infected children. Although control of rotavirus in adults, especially those immunocompromised due to HIV/AIDS remains a great challenge, vaccines have been introduced for prevention of rotavirus infection (Seheri *et al*, 2012). It is

expected with successful vaccination of infants, rota virus incidence will be reduced (Lopman *et al,* 2011). However, even with the high efficacy of the approved vaccines ranging from 89 to 98% in the industrialized world and parts of Latin America (Vesikari *et al,* 2006; Ruiz-Palacios *et al,* 2006, Linhares *et al,* 2008), a reduced efficacy ranging from 39 to 77% has been recorded in developing countries mainly in Africa and Asia (Zaman *et al,* 2010; Madhi *et al,* 2010; Armah *et al,*. 2010). Despite the diverse efficacy reported in developed and the developing countries (Madhi *et al,* 2010), strain diversity still remains a fundamental factor especially in ensuring efficacy of vaccines in this part of the world.

Many health centres have got no diagnostic capacity and even drugs to manage adult patients. Since rotavirus infection is self limiting supportive care is encouraged. Persistent diarrhoea affects up to 95% of persons with AIDS in developing countries. This frequently causes mal-absorption, significant weight loss (slim disease), high rates of extra intestinal opportunistic infections and increased morbidity. A study that was conducted in Lima,Peru, on aetiologies and manifestation of persistent diarrhoea in adults with H.I.V infection found out that *Cryptosporidium sp* (most predominant pathogen), *Giardia lamblia. Aeromonas SP, Campylobacter and* Rotavirus were significantly associated with persistent diarrhoea in H.I.V adults (Cárcamo *et al,* 2005). However a study that was conducted between H.I.V infected and non infected H.I.V children found out that there was no significant difference in rotavirus epidemiology as well as the rate of virus associated diarrhoea between the two groups (Liste *et al,* 2000). In conclusion, episodes of rotavirus diarrhoea in young children affect their physical growth further justifying the need of rotavirus immunization and other diarrhoea prevention measures (Natchu & Bhatnagar, 2013).

1.2 Statement of the Problem

There are three mechanisms through which rotaviruses diversify and evolve. Accumulation of point mutations generating genetic lineages that leads to emergence of antibody escape mutants. Genetic shift where there is exchange of genetic material via gene re-assortment during mixed infection of a single cell. Finally, introduction of animal rotavirus into human population (Kang *et al,* 2005).

Identification of rotaviruses with novel P/G combinations in Malawi emphasizes the ability of rotaviruses to undergo reassortment at high frequency. These may result in the formation of potentially new strains (Ramachandran *et al,* 1998). No much study has been conducted on characterization of rotaviruses circulating among children in developing countries (Cunliffe. *et al,* 1998) including the slum areas of Nairobi found in Kenya. Children living in slums are most at risk from contracting and dying from diarrheal illnesses because of poor sanitation and nutrition (Steele & Glass, 2011a). This group would benefit most from receiving vaccination against rotavirus. Thus it is important to investigate circulating serotypes with a view of assessing cross protection against all this rotavirus serotypes (Grimwood & Lambert, 2009).

In addition, a study carried out in Meru North district showed that G9 was the predominant genotype (Kiulia *et al,* 2006). A unique finding since strains G2-G4 which are known to be distributed worldwide were not found in any sample, with G1 accounting for only 17.4%. This is an indicator that different strains of the virus could be circulating in Kenya. It is therefore not clear whether the current Rotarix vaccine which contains G1 serotype (Phua *et al,* 2005) and the Rota Teq vaccine containing serotypes G1-G4 (Heaton *et al,* 2005) will provide cross protection against other emerging and unidentified strains in Kenya.

1.3 Justification

Even though the impact of rotavirus caused diarrhoea, as an opportunistic disease in HIV infection is known, very little information is available on the circulating strains within this group of patients, especially in Kenya where the latter continues to rise in number.

The fact that the strains that have not been known to infect humans are being isolated in high frequency among human subjects, justifies the need for continuous surveillance to determine circulating strains especially among infected children. Studies on the two capsid proteins (VP7 and VP4) in developing countries are essential in the continued vaccine studies since they are important in inducing protective immunity. In addition, because efficacy of the vaccines depends on identity between vaccine strain and prevailing wild type human rotavirus strains (Grimwood & Lambert, 2009). These will provide very important information if the

vaccines currently in use will succeed in preventing severe diarrhoea due to rotavirus infections and reduce the burden of hospital admissions.

1.4 Objectives

1.4.1 General Objective

To characterize human rotavirus group A serotypes causing gastroenteritis among children below five years of age and HIV-infected adults In Viwandani slum, Nairobi, 2013

1.4.2 Specific Objectives

1. To determine prevalence of rotavirus group A among children below five years of age and HIV-infected adults in Viwandani slum, Nairobi.
2. To determine the electropherotypes of rotavirus isolated among children below five years of age and HIV-infected adults in Viwandani slum, Nairobi.
3. To determine genetic diversity of rotavirus group A causing infections among children below five years of age and HIV-infected adults in viwandani slums,Nairobi

1.5 Study limitations

1. Lack of CD4 or HIV viral load in adults to conclusively link rotavirus infection to HIV infection
2. Further, we are unable to show whether there was any relationship between the adults in our study and the children; hence, the study cannot completely justify transmission within the groups
3. The study was also done within a small slum in Nairobi's urban setting and hence the findings cannot be extrapolated to cover strain diversity in the entire country

Despite these limitations, the weight of evidence in this study provides the most comprehensive data to date on rotavirus strains circulating in Viwandani slums, Nairobi in both children and H.I.V infected adults.

CHAPTER TWO
LITERATURE REVIEW

2.1 Rotavirus

Rotaviruses belong to the family *Reoviridae* and they are major cause of infantile diarrhea. Viral particle is up to 76.5 nm in diameter and is non enveloped (Patton, 1995). Rotavirus has seven major groups A-G (Al-Thani *et al*, 2013) most human strains belong to group A, although groups B and C have occasionally been associated with human illness (Iturriza *et al*, 2008)**.** Group A rotavirus (GARV) is the most important in human infections accounting for more than 90% of all rotavirus infections Santos & Hoshino (2005). The name rotavirus is derived from its wheel like appearance Anderson & Weber (2004).

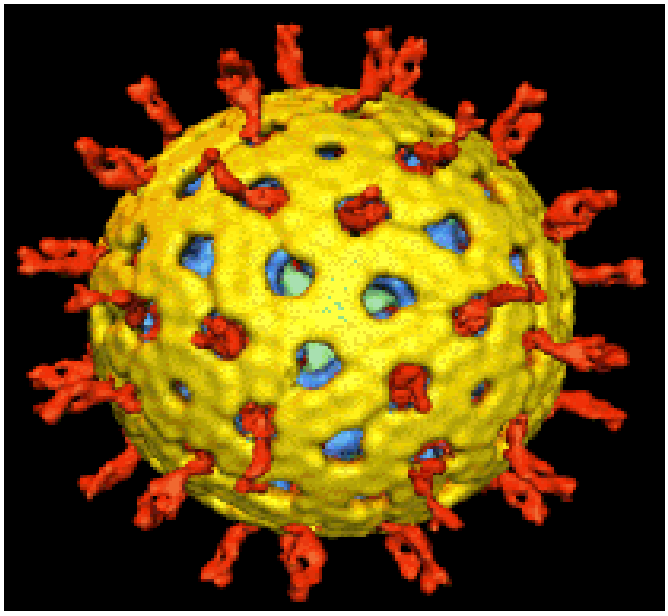

Figure 2.1: The wheel like appearance of rotavirus particle
(Umash et al, 2003)

Rotavirus strains are classified into two sub groups based on two structural proteins located on the surface (outer layer) of the virion. The glycoprotein VP7 defines G type while protease-sensitive protein VP4 defines P types (Iturriza *et al*, 2008). VP4 sare found on the surface spikes and are responsible for attachment on the host cell and virulence (Iturriza *et al*, 2008). G-types and P-types genes segregate independently of each other during reassortment Grimwood & Lambert (2009). There are at least 27 G types and 35 P types (Kiulia *et al*, 2014). The G serotypes 1–

4, and P genotypes P[8] and P[4] predominate worldwide (Ramachandran *et al,* 1998)

Rotaviruses have a segmented double stranded RNA genome consisting of 11 unique double helix molecules of RNA, which are 18,555 nucleoside base-pairs in total. Each helix, or segment is a gene numbered 1 to 11 by decreasing size. Each gene codes for one protein, except genes 9 and 11, which each code for two. The RNA is surrounded by a three-layered icosahedral protein capsid. Rotavirus genome codes for 6 viral structural proteins (VP1, VP2, VP3 VP4, VP6 and VP7) (Fig 2-2) and 6 non-structural proteins (NSP1-6) Grimwood & Lambert (2009)

The inner layer is formed by: VP2, which is the main structural component of the innermost layer involving the genome, the VP1, which is the RNA-dependent RNA polymerase for rotavirus, and VP3, which acts as the mRNA capping enzyme (guanilyltransferase and methylase) and is a replication intermediate (Vende *et al,* 2002)

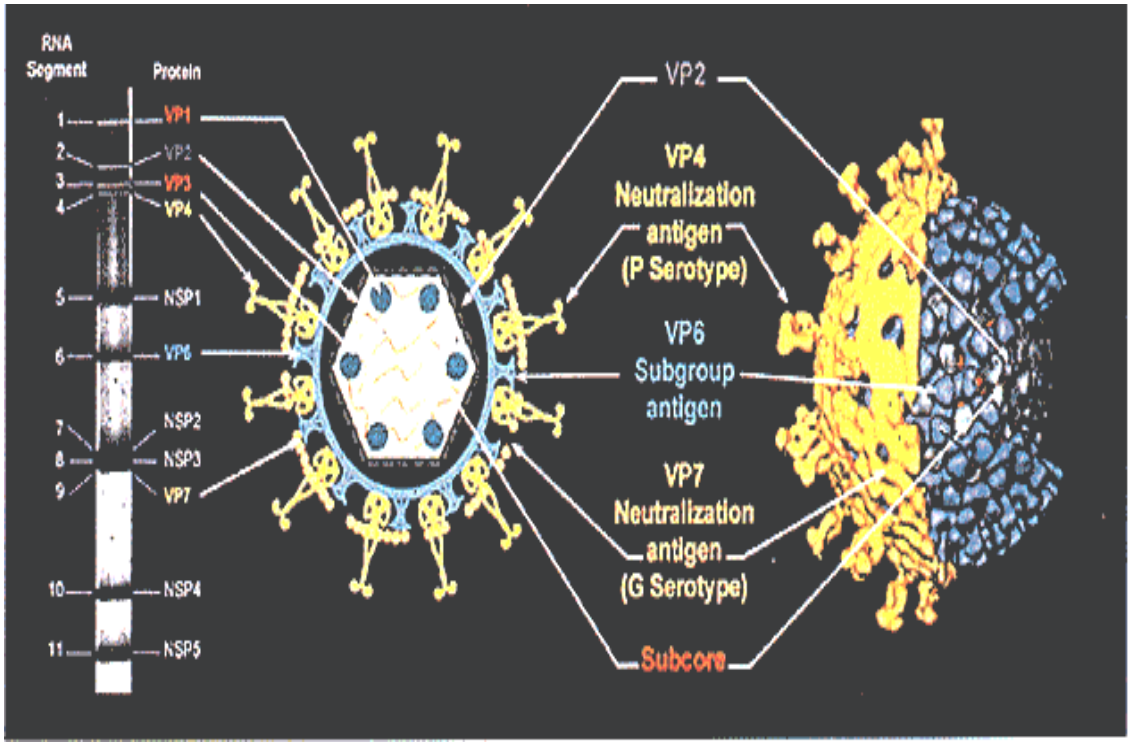

Figure 2.2: Structural diagram of Rotavirus

The intermediate layer is made up of the structural VP6 associated with VP2 and confers to the structure of the so-called double-layered particles (DLP). The outer

layer is constituted by trimeric structures of VP7 glycoprotein and the dimeric spikes of VP4 forming the triple-layered particles (TLP); the infectious form of the virus (Grimwood & Lambert (2009). These two structural proteins, VP7 (the glycoprotein or G protein) and VP4 (the protease-cleaved protein or P protein), make up the outer shell and are considered important for vaccine development. In addition, these define the serotype of the virus and major antigens involved in virus neutralization (Grimwood & Lambert (2009).

2.1.2 Rotavirus Non-Structural Proteins

Table 2.1: The functions of the non-structural proteins of rotaviruses.

Non-Structural Protein	Function
NSP1–	Binds Interferon Regulatory Factor 3 and may inhibit interferon response during rotavirus infection (Grimwood & Lambert (2009).
NSP2–	In conjunction with NSP5, NSP2 is involved synthesis and packaging of viral RNA and creation of viroplasms. The protein is a replication intermediate (Umash *et al*, 2003). .
NSP3 –	Is a 36kD protein that binds viral mRNA at the 3' end and promotes viral protein synthesis. It also represses host cell protein synthesis. This protein is a possible target for a new class of antiviral (Grimwood & Lambert (2009) .
NSP4 –	Has been shown to act as enterotoxin and cause diarrhoea during infection. There is also correlation between VP6 virus subgroup and NSP4 genotype (Umash *et al*, 2003). .
NSP5 –	This phosphoprotein works with NSP2 in RNA synthesis and packaging, and to induce viroplasms. It is also a replication intermediate (Umash *et al*, 2003). .
NSP6 –	Little information is available on NSP6, but it is associated with NSP5 and its function (Umash *et al*, 2003).

2.2 Laboratory Methods for the Detection of Rotavirus Strains

Various methods have been used to detect rotaviruses.

2.2.1 Rotavirus Culture Methods

Despite the high quantity of virus particles shed in animal faeces and high rate of

rotavirus infection, rotaviruses have proved difficult to grow in cell cultures (Estes. *et al,* 1979). Successful propagation of rotaviruses has been achieved by use of Vero. Other culture methods employ requirement of calcium and 5% chicken serum to aid in efficient multiplication of the virus (Hasegawa *et al,* 1982; Sato *et al,* 1981). Nevertheless, different rotavirus strains vary in their capacity to grow in cell cultures and growth of rotavirus from clinical faeces is very difficult and does not work for every sample (June and Hall.T 1978). Bacterial contamination can be prevented by centrifugation and use of antibiotics in the culture throughout during infection. Cyto-pathogenic effects may also not be visible following infection in African Green Monkey Kidney Cells (AGMK). In addition the faeces may be cytotoxic and dilution required before infection. Rhesus Monkey Kidney (MA104) cells are commonly used for propagation and characterization of both animal and human rotaviruses. Despite this knowledge about factors necessary for virus propagation the rate of isolation of rotavirus in cell culture is very minimal hence this is not a suitable method for rotavirus detection.

2.2.2 Rotavirus Electron Microscopy

Electron microscopy was traditionally used for rotavirus detection. This technique is advantageous because of high speed, simplicity, high resolution and good preservation of three dimensional structures of virus particles However this method is insensitive as it requires 10^8 particles concentration for virus detection (Iturriza *et al,* 2008). In addition, this technique is not only labor intensive for detection of large numbers of stool specimens but the technique also requires highly trained personnel nevertheless this technique cannot distinguish between different groups of rota virus. Moreover electron microscope is a very expensive instrument to purchase (Cárcamo *et al,* 2005).

2.2.3 Immunological techniques for Rotavirus Detection

Antigen detection methods like enzyme immunoassays (EIA) latex agglutination, lateral flow immunoassays and immunochromatography have been employed. They detect protein antigens on rotavirus particles in stool specimens. ELISA that uses rotavirus antibodies to capture antigens onto wells of plastic plates and detected in

colorimetric reaction by a second rotavirus antibody coupled to an enzyme is widely used. ELISA is very sensitive, specific and is important in detecting large volumes of stool samples (World Health Organization, 2009). It is more preferably used because it is convenient, reliable and inexpensive. The major disadvantage of this assay is the inability to be able to detect non group A rotaviruses. Latex agglutination utilizing latex particles coated with anti rotavirus antibodies can be used for rotavirus antigen detection. The LA technique has lower sensitivity than EIA. ICG shows high sensitivity and results comparable to those achieved with EIA, and is rapid and technically very simple (World Health Organization, 2009). Immunochromatographic methods are being widely employed for rapid testing.

2.2.4 Molecular Identification of Rotavirus

Nucleic acid amplification by PCR of VP7 and VP4 rotavirus genes has been recently developed for detection of rotavirus. This method is 1000 times more sensitive than immunoassays in detection of rotavirus and other enteric viruses (Iturriza *et al,* 2008). These two genes in rotavirus genome are very important not only for surveillance studies to determine circulating strains but also for vaccine development (Kapikian *et al,* 1996). Real time PCR is useful for verifying that RNA extracts contain intact rotavirus RNA. This technique is very expensive, labour intensive and not suitable for routine rotavirus detection studies (World Health Organization, 2009).

2.2.5 Electropherotyping of Rotaviruses

Electrophoresis has been used as a powerful tool for rotavirus detection. Polyacrylamide gel electrophoresis allows classification of rotavirus into long and short electropherotypes based on migration pattern of gene segment on acrylamide gel. Rotavirus electropherotypic patterns can be visualised after RNA extraction from stool samples on acrylamide gels by electrophoresis, and staining with ethidium bromide and silver nitrate (World Health Organization, 2009).

Human rotavirus Groups A, B and C have distinct patterns of gene distribution. The electropherotypes correlate with the presence of viruses of specific groups A, B and C. For example for Group A rotavirus they will have characteristic patterns like in (Fig 2.3). polyacrylamide gel electrophoresis has the same sensitivity as that of enzyme immune assay methods (Herring *et al*, 1982). PAGE has been widely used to detect rotavirus infections in surveillance studies however; this method is labour intensive and time consuming (World Health Organization, 2009).

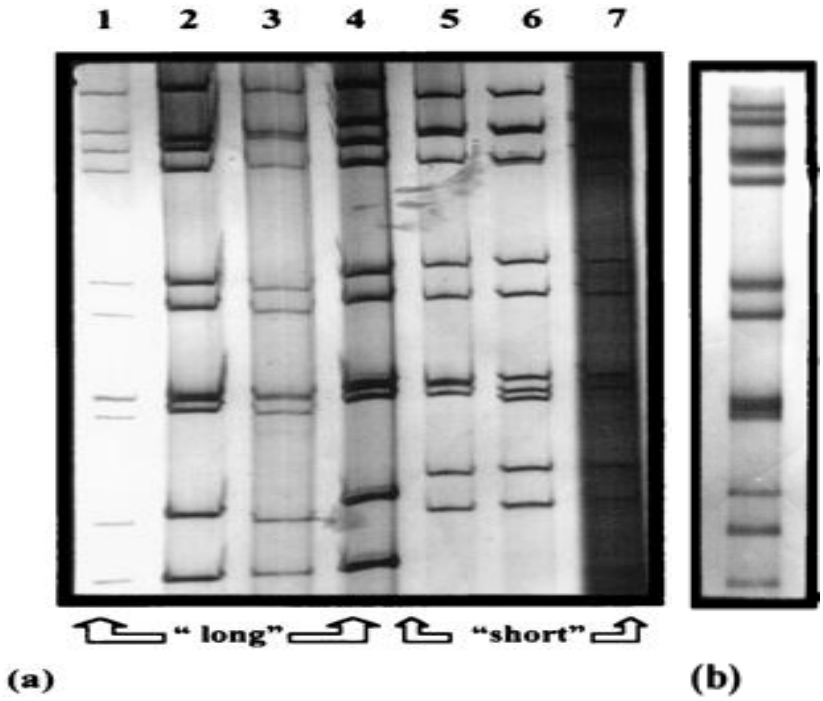

Figure 2-3: Rotavirus electropherotypes.
(a) Rotavirus electropherotypes. 1, 2, 3 and 4 are long electropherotypes while 5,6 and 7 are short electropherotypes. (b) mixed infections (Matsuno *et al*, 1985).

2.3 Pathogenesis

Rotavirus spreads from person to person, mainly by faecal oral transmission. After ingestion, rotavirus particles are carried to the small intestine where they enter matureenterocytes (Holmes *et al*, 1975) through either direct entry or calcium dependent endocytosis (Lundgren O & Svensson L 2001). After cytolytic replication in the mature enterocytes of the small intestine, new rotavirus particles can infect distalportions of the small intestine or be excreted in the faeces. More than 1010–1011 viral particles per gram of faeces are excreted by children during infection

(Desselberger U, 1999). The amount of rotavirus excreted by adults might be more variable. In at least one study, shedding was 10–100-fold lower in travellers' diarrhoea (Vollet, J. *et al*, 1979 Symptom-free adults can shed rotavirus in quantities so low as to be undetectable by most routine assays (Barnes *et al*, 2003)

The mechanism by which rotavirus induces diarrhoea is poorly understood. Few investigations have incorporated the study of human mucosal samples. The reports that are available describe various findings: villous shortening, flattening, and atrophy, denudation of microvilli, mitochondrial swelling, distension of the endoplasmic reticulum, depressed disaccharidase concentrations, and infiltration of mononuclear cells (Lundgren O and Svensson L 2001); Barnes *et al*, 2003) Additional hypotheses about the pathophysiology of rotavirus gastroenteritis have been generated from animal studies. In one review the diminished ability of the intestinal epithelium to absorb fluid and nutrients (Lundgren. O & Svensson. 2001). Stimulation of the enteric nervous system, (Lundgren *et al*, 2000) and local villous ischaemia and shortening resulting in impaired nutrient absorption were noted. A murine model of rotavirus infection suggests that rotavirus NSP4 acts as an enterotoxin, potentially by increasing calcium-dependent signalling of chloride secretion (Horie. Y. *et al*, 1999).

2.4 Transmission of Rotavirus

Rotavirus gastroenteritis is transmitted via faecal-oral route although to some lesser extent it might be caused through respiratory transmission (Midthun, 1996). The low infection dose of (10-100 virus particles) makes it difficult for good hygiene practices to prevent the spread of gastroenteritis diarrhoea (Steele & Glass, 2011a). There are various mechanisms through which rotavirus cause gastroenteritis diarrhoea. These mechanisms include reduced absorptive surface, invasion of enterocytes, villus ischemia and impairing absorption through cellular damage (Lundgren and Svensson, 2001). Rotavirus infections can be diagnosed via symptoms like mild fever accompanied with profuse watery diarrhoea, vomiting, and abdominal pain. Severe cases cause death through dehydration (Elhag *et al*, 2013).

2.5 Treatment and Prevention of Rotavirus Infections

2.5.1 Treatment

Management of rotavirus gastroenteritis symptoms in order to restore the normal physiological function of the body is essential. Preventing dehydration is the most important step in treatment of viral gastroenteritis. Therefore, liquid intake in order to correct the water deficit due to vomiting and diarrhoea is necessary. This is achieved through administering oral rehydration therapy (Kapikian *et al,* 1996). Administration of zinc and oral rehydration salts which are commercially manufactured are the mainstay of rotavirus infection treatment that has proved to reduce high rates of mortality (Natchu & Bhatnagar, 2013). However, adults can be encouraged to drink fluids to curb down dehydration.

Antiemetic drugs may also be helpful for vomiting in children (Haffejee, 1991). Lactobacillus species bacteria have been administered in children although in very rare occasions to reduce duration of diarrhoea. However, if the symptoms persist and patient becomes dehydrated hospital admission of intravenous fluids is necessary.

2.5.2 Prevention

Since rotavirus infection is facilitated through oral-faecal route, various factors like avoiding contaminated water and food should be kept in place. In addition washing of hands should be necessary since 43% of virions of rotavirus are able to survive in human fingers for up to 60 minutes (Ansari *et al,* 1988). Protective clothing like gloves, gowns, frequent hand washing should be practiced in care of patients infected with rotavirus infection. (Mofenson *et al,* 2010) reported that low humidity favour survival of rotavirus on non-porous surfaces at room temperature. Use of hypochlorite disinfectants and use of 70% ethanol can deactivate rotavirus virions (Springthorpe *et al,* 1986). Development to rotavirus vaccines due to high rates of morbidity and mortality associated with rotavirus infections has been developed (Kang *et al,* 2005). Rotavirus vaccine development is based on the necessity for a polyvalent vaccine comprising of the epidemiologically group A rotavirus serotypes (Gouvea *et al,* 1990). The rhesus rotavirus tetravalent vaccine was formulated to confer immunity against four serotypes that predominate globally (Ramachandran *et*

al, 1998). Most frequently occurring G-types (G1–G4) have been used in the development of a vaccine, and also the most occurring P-type found in human rotaviruses (P8) has been included. Monovalent Rotarix vaccine (GlaxoSmithKline, Research Triangle Park, NC, USA) and pentavalent Rotatek vaccine (Merck, Rahway, NJ, USA), which are oral live vaccines have reduced considerably hospitalizations and clinic visits (Grimwood & Lambert, 2009). These vaccines have proved to be effective and safe (Gouvea *et al*, 1990). Nevertheless, there are several challenges facing these vaccines in reducing childhood mortality, clinic visits and hospital admissions. It is yet to be determined whether these vaccines will be able to provide cross protection against rotavirus caused gastroenteritis in developing countries. Early vaccines were less immunogenic in tropical countries and they failed to provide protection in Africa (Grimwood & Lambert, 2009).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Design

This was a cross-sectional, laboratory-based descriptive study.

3.2 Study Setting

Viwandani slum is located 7kms from Nairobi city centre and has close proximity to the city's industrial area. It's one of the most congested slum areas in Nairobi with over 250 dwelling units per hectare. The slum is characterized by poor sanitation and waste disposal.

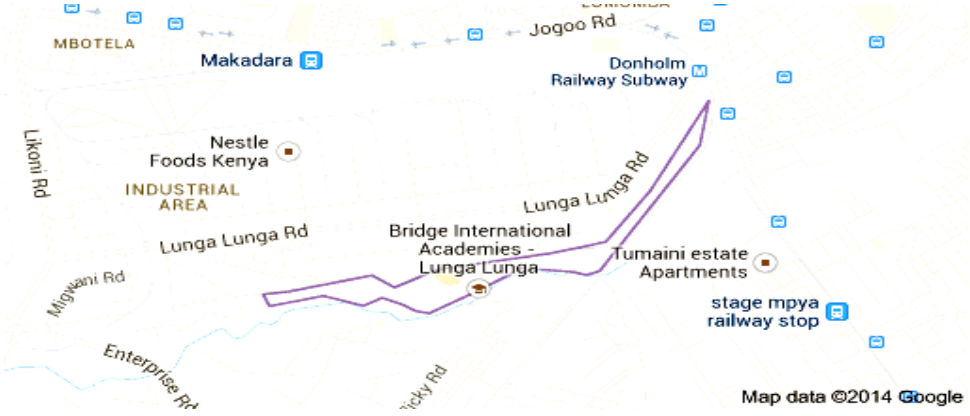

Figure 3.1; Map of Viwandani in the industrial area of Nairobi
(Source: Google Maps, accessed May, 2015).
The map shows the position of Viwandani slum within Nairobi County in Kenya.

3.3 Study population

Children below 5 years of age and HIV antibody positive adults presenting with symptoms of gastroenteritis were recruited in this study.

3.3.1 Inclusion criteria

<u>Children</u>

- All children regardless gender aged five years and below presenting with diarrhoea not exceeding 7days.
- Consent of parents or guardian
- A child diagnosed with acute diarrhoea

Adults

- Must be HIV-positive
- Aged 18 years old and above presenting with acute diarrhoea
- Consenting subjects.

3.3.2 Exclusion criteria

Children

- All children above the age of five years with diarrhoea
- All children aged five years and below with diarrhoea exceeding 7days.
- Non- consent of parents

Adults

- HIV negative or those whose HIV status are not ascertained
- Adults Presenting with bloody diarrhoea
- Adults Presenting with symptoms for more than 48 hours
- Adults diagnosed of other diseases apart from diarrhoea-related
- Non-consenting individuals

3.4 Sample size determination

Rotavirus caused gastroenteritis in Kenya has got an estimated prevalence of 22% in children (Nakata *et al,* 1999). At 95% confidence interval, a minimum sample size of 260 samples was collected for analysis of both children and H.I.V positive adults. The size was obtained at per the statistical formula (Cochran, 1963):

$$n = \frac{Z^2\ 1\text{-}\alpha/2P(I\text{-}P)}{d^2}$$

Where

n = Minimum sample size

$Z_{1\text{-}Mi}$= Standard error for confidence interval at 95% = (1.96)

P= Estimated prevalence for rotavirus infection among children in Kenya (22%)

d= Precision of the estimate required (1-α= 0.05

A total of 128 stool samples from infants and children below 5 years and further 132 from HIV positive adults were collected for the study. HIV positive adults were those whose HIV status had been established

3.5 Sampling Method

Consecutive sampling of every person that met the inclusion criteria was applied until the required sample was achieved. Recruitment of the patients in the study was integrated within the day-to-day activities of the clinic. The clinician explained to the patients in either English or Kiswahili and an informed consent (Appendix 1-IV) was obtained from those who agreed to participate.

3.6 Faecal specimen collection and handling

Faecal samples were collected from 260 outpatients (128 children below 5 years and 132 HIV positive adults) who visited Mukuru kwa Njenga and Mukuru kwa Reuben health canters in Viwandani slum presenting with gastroenteritis symptoms, from August 2012 to July 2013. Faecal specimens were collected into sterile containers by patients. Rotavirus dipstick rapid kit was used to test faecal specimen within 48 hours. Patients whose results were positive were treated immediately. Specimens were transported to the Virology Laboratory in Kenya Medical Research Institute, in sterile containers placed in a cool box at negative 4 and stored at -20 °C in Centre of Virus Research in KEMRI to wait processing.

3.7 Laboratory Procedures

3.7.1 Specimen Processing

Stool samples were received in sterile polypots from patients in two health centres. They were placed in a cool box that had ice bags at temperature of four. Later they were transported to KEMRI headquarters centre for viruses research in rotavirus laboratory. A pea size (approximately 10g) of solid stool or 700µl of loose stool aliquots was made into cryovials. Finally they were stored in refrigerators at -20°C to wait processing once the sample size was achieved.

3.7.2 Rotavirus ELISA

Before starting ELISA faecal suspensions were made by adding 5 Ml of the prospect rotavirus sample diluents to 0.1g of solid faeces (small pea sized portion) or approximately 100µl of liquid faeces using transfer pipettes.

Including a negative and positive control, 100µl of diluted faecal suspension was added to micro wells in order to separate. A known positive sample for ELISA and water was used for both positive and negative control respectively. To each microwell, 100µl of the conjugate was added. The immuno plate was sealed with plate sealer and incubated at room temperature for one hour. Faecal supernatant was then aspirated and the plate washed five times with diluted buffer. The immune plate was being blotted after each was on clean absorbent towel to remove excess buffer. The substrate was then added to each microwell and the plate covered and incubated at room temperature for 10 minutes. The reaction was stopped by addition of stop solution to each microwell. Optical densities results of each microwell were read using a spectrophotometre at 450nm. Cut-off value was calculated by adding negative control + 0.2. Any sample whose optical density exceeded the cut off value was considered positive.

3.7.3 Polyacrylamide Gel Electrophorensis (PAGE)

PAGE was carried out in three steps namely, extraction of rotavirus ds RNA from stool samples, gel casting and silver staining. Unknown long and short strain positive samples were used as controls.

3.7.3.1 Extraction of rotavirus ds RNA by Phenol Chloroform Method

A pea sized amount of faecal material was added to 5 ml of distilled water to make stool suspension and they were mixed well by vortexing. Into a clean eppendorf tube 50 µl of prewarmed sodium acetate containing 1% sodium dodecyl sulphate (SDS) was added. The mixture was incubated in a 37°C water bath for 15 minutes. After the incubation, 500 µl of phenol chloroform was added to the eppendorf and vortexed for 1 minute and incubated further for 15 minutes in 56°C water bath. The eppendorf tubes were then opened and resealed to reduce air pressure within the tube and to prevent the tubes from popping open during mixing. They were then vortexed for 1 minute and centrifuged for 2-3 mins at 12000 xg. The upper aqueous phase containing the ds RNA was carefully removed to avoid any interface material as this contains protein and DNA that will contaminate the extraction and potentially degrade the RNA and put in a clean eppendorf tube. 40µl of 3M NaAc and 1M ice

cold absolute ethanol was added to the sample and mixed gently by turning the tube 4-6 times and incubated at -20°C for 16 hours to allow the ds RNA to precipitate. They were then centrifuged at -4°Cfor 15 mins at 12000 x g.

To pellet ds RNA and the supernatant was poured off and the samples were allowed to air dry finally, the pellet was re-suspended in 30µl PAGE sample dye before loading on a PAGE gel.

3.7.3.2 GEL CASTING.

The rotavirus dsRNA was run on polyacrylamide gels using a large-format gel electrophoresis system (Hoefer SE600) followed by silver staining to group the Rotavirus.

Ethanol was used to clean thoroughly glass plates and assembled for gel casting. 10% resolving gel was made by adding 15µl of (8.9) TEMED, 15.8 ML of distilled water , 10 ML of 3.5% acrylamide stock and 450µl of 10% of ammonium persulphate into a conical flask and mixture poured into the assembled equipment of gel casting and left to polymerize for 1 hr. A 3% spacer gel was then made by adding 6.8ml of distilled water, 1.6ML of 30% acrylamide stock, 1.25 ML of pH 8.9 of resolving buffer, 5µl of TEMED and 10% of ammonium persulphate into a conical flask. This spacer gel was then poured into the gel casting equipment and a comb was then inserted to form wells. This then was also left to polymerize for 45 mins. The comb was then removed and samples that were suspended in 30µl PAGE dye loaded on PAGE gel.

3.7.3.3 SILVER STAINING.

The equipment for gel casting was unassembled and the gel was removed from the glass plates. It was then cut at the bottom of the right hand corner to orientate it. Fixing solution one was then added to the gel and incubated for 30 mins on an orbital shaker. Fixing solution one was then drained and replaced with fixing solution two and was incubated for 30 mins on the orbital shaker. Fixing solution two was then drained and silver nitrate staining solution was added and incubated for 30 minutes on the orbital shaker. Silver nitrate solution was drained and the gel washed using

distilled water for two minutes. 50 ML of developing solution added to the gel to remove any black precipitates. The 50 ml developing solution was drained off and another 200ML developing solution was added and incubated for 5 mins till RNA bands were seen. The developing solution was then drained and stopping solution added to prevent further colouring. The gel was then rinsed well in distilled water and visualised on an illuminator to view the bands.

3.7.4 R T-P C R of Rotavirus dsRNA (VP4 and VP7 cDNA synthesis)

The presence of viruses in stool samples was determined using Polymerase Chain Reaction (PCR) technique. An established WHO Rotavirus Reference Laboratory Manual 2002 (South Africa) was used. Genotyping involved extraction of dsRNA by trizol method, reverse transcription of purified RNA, first round polymerase chain reaction of cDNA, and a nested PCR of the amplified VP4 and VP7 genes.

3.7.4.1 Extraction of dsRNA by Trizol Method

Purified rotavirus double stranded RNA was extracted using Trizol method in the WHO rotavirus reference laboratory manual 2002 (South Africa). Stool suspensions were made by adding a pea-sized amount of fecal material to approximately 5ml of distilled water and mixed well. The suspensions were then centrifuged at 5000xg for 5 minutes at room temperature after which 200µl of the supernatant was transferred to a clean eppendorf tube. 500µl of Trizol were added to the tubes and vortexed for 30 seconds, and then incubated at room temperature for 5 minutes. To the tubes, a 100µl of chloroform was added and vortexed for 30 seconds and then incubated at room temperature for 3 minutes. The tubes were centrifuged at 12000 rpm for 15 minutes at 4^0C to separate the phases.

The clear aqueous phases were transferred to clean eppendorf tubes avoiding the white interface and pink organic phase. 700µl of ice-cold isopropyl alcohol were added and mixed gently by turning the tubes 4-6 times. They were then incubated at room temperature for 20 minutes. The tubes were centrifuged at 12000xg for 15 minutes at 4^0C to pellet the dsRNA. The supernatant was discarded and the pellets

allowed to air dry. The pellets were then resuspended in 15µl of de-ionized water awaiting reverse transcription.

3.7.4.2 Reverse Transcription

Reverse transcription was done using RevertAid^{TM} First Strand cDNA Synthesis kit (Seegen, South Africa) following the manufacturer's protocol below.

Protocol

The following reagents were added to an RT tube on ice; 8µl of total dsRNA, 1µl of random hexamer (0.2µg/ µl) and 3µl of DEPC–treated water, adding up to a total volume of 12µl per tube. The tubes were then incubated at 80^{o}C for 3 minutes and latter chilled on ice for 2 minutes after which they were span briefly.

The following reagents were then added to the tubes; 5µl of 5x RT buffer, 2µl of 10mM dNTP, 1µl of RNAse inhibitor (20 u/µl) and 1µl of reverse transcriptase (200 u/µl) adding up to a volume of 20µl per tube. The tubes were then incubated at 37 ^{o}C for 90 minutes and then heated to 94^{o}C for 2 minutes, after which they were chilled on ice for 2 minutes and later span briefly. All the cDNA samples were stored at -20 ^{o}C until ready for use.

3.7.4.3 Amplification of cDNA by multiplex PCR

Amplification of cDNA was done using Seeplex® Diarrhoea ACE Detection kit (Seegen, South Africa) according to the manufacturer's protocol illustrated below. This kit allowed a multiplex assay that permitted the simultaneous amplification of target cDNA of human Group A rotaviruses and an internal control (IC).

A mater mix which constituted 4µl 10mM dNTP's, 0.3µl *Taq* Polymerase, 4µl x10 *Taq* Buffer, 2.4µl 25mM $MgCL_2$ and 30µl dH_20 was prepared whereby, the volume of each reagent was multiplied by the number of samples. 40µl of the master mix were then put into each tube containing cDNA and span down briefly before placing in PCR block.

They were then ran using the following program (Table 3.0).

Table 3.1: Thermo cycler program for amplification of cDNA

Cycling profile	No. of cycles	Temperature	Duration
Denaturation	30	95^{o}C	30
Annealing	30	42^{o}C	30
Elongation	30	72^{o}C	1 min
Final elongation	30	72^{o}C	7 min

After amplification, the samples were then viewed under UV light in a 1% agarose gel alongside a 100 bp ladder.

3.7.4.4 Genotyping of rotavirus using VP4 and VP7 specific primers

Consensus primers sBeg 9 (GGCTTTAAAAGAGAGAATTTC) - position 1-21 and End 9 (GGTCACATCATACAATTCTAATCTAAG) – position 1062-1036 while P typing consensus primers con3 (TGGCTTCGCCATTTTATAGACA) - position 11-32 and con2 (ATTTCGGACCATTTATAACC) – position 868-887 for Group A rotaviruses were used (Gouvea *et al,*1990; Gentsch *et al,* 1992).

After extraction, 8µl of each purified dsRNA were put in a clean PCR eppendorf tube and 1µl of each transcription primers added (sBeg and End9 for VP7, and Con2 Con3 for VP4). The mixture was then boiled for 5 minutes and immediately chilled in an ice bath. A mater mix (which constituted 0.8µl, 10mM, dNTP's, 0.4µ RTase (AMV) and 2.0µl 5x AMV buffer) was prepared whereby, the volume of each reagent was multiplied by the number of samples. 3.2µl of the master mix were then put into each tube containing denatured RNA and incubated in a water bath at 42^{o}C for 26 minutes.

3.7.4.3 Genotyping (Nested PCR of the amplified VP7 and VP4) genes

3.7.4.3.1 VP7 nested PCR

A master mix was prepared by adding 10mM dNTP's, 25mM $MgCL_{2,}$ x10 *Taq* Buffer, *Taq* Polymerase, dH_20, primers of each VP7 genotype ($G_{1,}$ $G_{2,}$ $G_{3,}$ $G_{4,}$ $G_{8,}$ G_9) , primer sBeg to a clean eppendorf tube, while multiplying the volume of each reagent by the number of the samples. To ensure quality of the results negative control where

primers were not added to one eppendorf tube was used. 40µl of the master mix were put into each tube containing the first time amplified VP7 cDNA and ran for thirty cycles in a thermo cycler. The samples were then ran in 1% agarose gel and viewed under UV light alongside 100 base pair ladder**.** The table 3.1 below shows the oligonucleotide primers used for VP7 genotyping.

Table 3.2: Oligonucleotide primer for G-typing by

Genotype	**Sequence (5'-3')**	**position**	**primer**
G1	CAAGTACTCAAATCAATGATGG	314-335	aBt1
G2	CAATGATATTAACACATTTTCTGTG	411-435	aCT2
G3	CGTTTGAAGAAGTTGCAACAG	689-709	aET3
G4	CGTTTCTGGTGAGGAGTTG	480-498	aDT4
G8	GTCACACCATTTGTAAATTGG	178-198	aAT8
G9	CTAGATGTAACTACAACTAC	756-776	aFT9

(Gouvea *et al,* 1990 and Gault *et al,* 1999

3.7.4.3.2 VP4 nested PCR

A master mix was prepared by adding 10mM dNTP's, 25mM $MgCL_2$, x10 *Taq* Buffer, *Taq* Polymerase, dH_20, primers of each VP4 genotype (P_4, P_6, P_8, P_9, P_{10}) and the Con_3 primer to a clean PCR eppendorf tube, while multiplying the volume of each reagent by the number of the `samples. To ensure quality of the results negative control where primers were not added to one eppendorf tube was used. 40µl of the master mix were then put into each tube containing the first time amplified VP4 cDNA and then transferred to a thermo cycler that had been set to run 30 cycles. The samples were then run in a 1% agarose gel and viewed under UV light alongside 100 base pair ladder. Primer in table 3.2 were used for VP4 genoyping

Table 2 Table 3.2; Oligonucleotide primer for P-typing

Genotype	Sequence (5'-3')	position	primer
P4	CTATTGTTAGAGGTTAGAGTC	474-494	2T-1
P6	TGTTGATTAGTTGGATTCAA	259-278	3T-1
P8	ACTTGGATAACGTGC	339-356	1T-1
P9	TGAGACATGCAATTGGAC	385-402	4T-1
P10	ATCATAGTTAGTAGTCGG	575-594	5T-1

(Gentsch et al, 1992)

3.8 Data Management

Patients in the study were identified with their laboratory numbers. Data obtained from each patient in the study was entered into a patient register after which the data was entered into a spread sheet where the data was be stored and protected using passwords known only to the principal investigator

3.9 Data Analysis and Presentation

The data collected was entered into a database created in Microsoft excel and was subjected to explanatory data analysis and all statistical analysis was done using the chi squire. The prevalence of rotavirus age, gender and seasonal distribution were presented as proportions of the total population.

3.10 Ethical Considerations

This study was approved by Ethical Review Committee (ERC) of Kenya Medical Research Institute (KEMRI). It was voluntary and parents or guardians signed a concept document to allow participation of their children in the study. Personal identification information was removed from the faecal samples for viral gastroenteritis analysis, and the samples were assigned new laboratory numbers.

This study did not involve any invasive procedure. The patient collected the stool in the containers in the normal defecation process. This study therefore did not subject the patient/ subject to any pain.

Subjects had direct benefit since they were tested for rotavirus in the clinic using rotavirus rapid kit. Also the results of the study has been shared with KEMRI and other relevant authorities, who will take measures to advice the general public, based on the results, and take the necessary course(s) of action. The results obtained also have further provided information on circulating strains in the region.

CHAPTER FOUR

RESEARCH FINDINGS and DISCUSSION

4.1 Prevalence of Study Participants

A total of 260 faecal specimen samples were collected for analysis (128 from children and 132 from HIV infected adults). All specimens were first subjected to Enzyme Immunosorbent Assay (ELISA) screening. A total of 30 (23%) specimen from children and 10 (8%) from adults were detected positive by ELISA (AppendixV and VI). Analysis of detections in children < 5 years based on age distribution, recorded the highest prevalence within the age group between 7 to 12 months followed by those within the age group of 13 to 24 months. The least prevalence was recorded for the group of <1 to 3 months (Figure 4-1). In the HIV antibody positive adults (Figure 4-2), rotavirus infection was realized in high frequency in the group of 49 to 58 years.

4.1.2 Age of patients with rotavirus diarrhea

The proportional age distribution of children less 5 yrs and HIV positive adults with acute viral diarrhoea as diagnosed by ELISA is shown in figure 4.1 and figure 4.2 below.

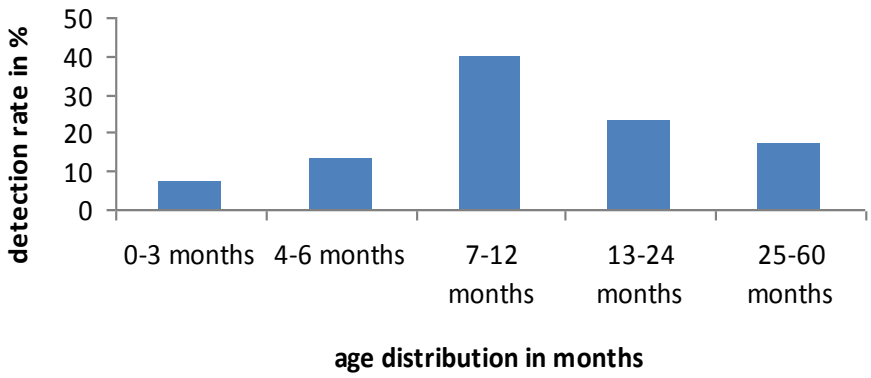

Figure 4.1: Age distribution of rotavirus infection in children below 5 years

The figure shows a high rate of infections between the ages of 7 to12 with a significantly lower rate between the ages of 0 to 3 and 25 to 60 months

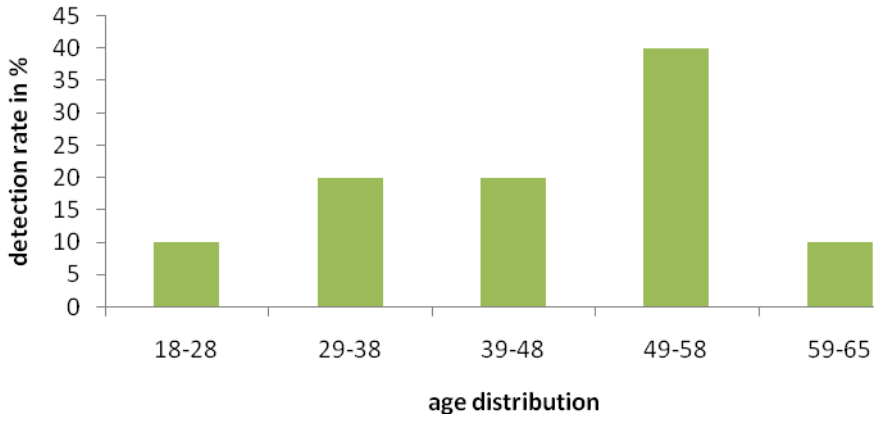

Figure 4.2: Age distribution of rotavirus infection in HIV infected adults

The figure shows the high rate of infections in older adults above 49 years as compared to young adults of age 18 to 28 years

4.1.3 Distribution pattern of rotavirus infection in adults and children

Analysis of distribution by gender was performed using Chi square test to determine the difference in distribution patterns of rotavirus infection. P< 0.05 was considered significant.

The results showed no significance in distribution of rotavirus infection in the two groups based on gender. However, the results showed statistical significance in distribution of rotavirus infection between children and adults based on gender as shown in table 4.1 below.

Table 4.1: Distribution of rotavirus infection among children and adults.

Patient category		**Outcome**		**P. value ***
		Positive	Negative	
Adults		10	122	0.0002
Children		30	98	
Adults	Male	3	42	1.0000
	Females	7	80	
Children	Male	14	50	0.8350
	Females	16	48	

*value < 0.05 considered significant

The results in the table indicate significance difference in rotavirus infection distribution between children and adults

4.1.4 Monthly distribution of rotavirus infection

This study found out that rotavirus was in circulation all year round however, rotavirus incidence was high during wet and cold months of March April and November and dry month of August as shown in figure 4.3 below.

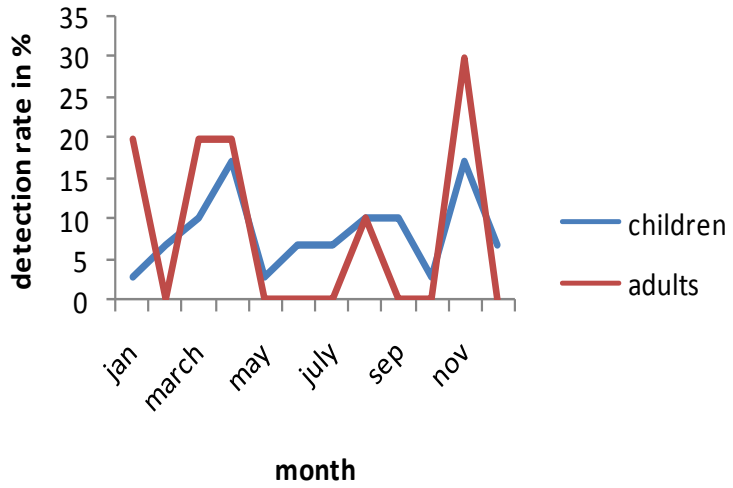

Figure 4.3: Monthly distribution of rotavirus infection in children below 5 yrs and HIV positive adults

This figure shows that rotavirus incidence was high during wet and cold months of March, April and November and dry month of august

4.2. Percentage Isolated rotavirus electropherotypes

Two different rotavirus strains as defined by PAGE-RNA electropherotypes co-circulated in the area, with the long strain being predominant and persistent during the period of this survey as shown by figure 4.4 below.

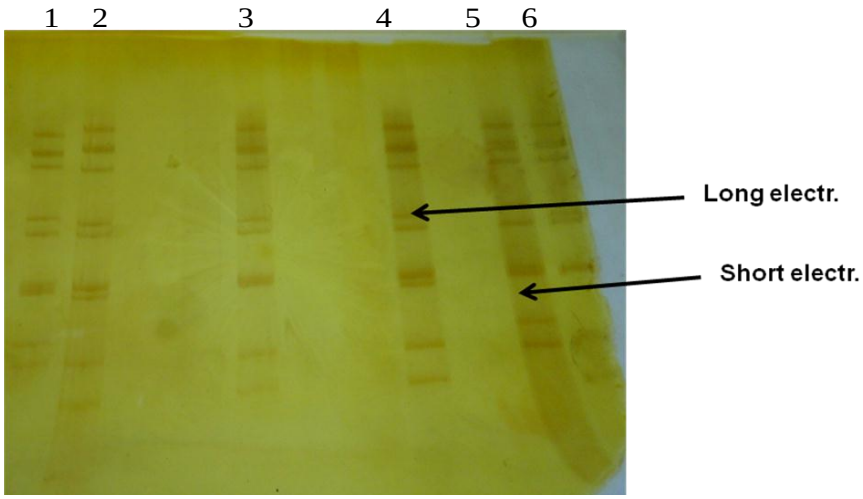

Figure 4.4: An acrylamide gel under UV light.

Lane 1 is the unknown positive short strain control used in the experiment, lanes 2, 3, 4 and 6 are long strain electropherotypes while lane 5 is a short electropheroype. Short strains are identified by the short distance of RNA migration on the gel while long strains are identified by the longer distance of RNA migration on the gel.

Analysis of electropherotypes revealed that 80% (24) of the isolated strains were long electropherotypes while 20% (6) of the strains were short electropherotypes among the children. Long electropherotypes accounted for 60% (6) and short accounted for 40% (4) in adults. No mixed infection was detected within the electropherotypes

It was also realized that the long electropherotypes did not appear to vary with age among children and occurred in all ages. Short electropherotypes on the other hand were identified only in children < 2 years old (Table 4.2).

Table 4.2: Distribution of rotavirus RNA electropherotypes among children

	Electropherotypes	
Age groups	Long patterns	Short patterns
0-3 Months	2	0
4-6 Months	3	0
7-12 Months	10	3
13-24 Months	4	3
25-60 Months	5	0

This table shows that long electropherotypes occurred in all ages in among children while short electropherotypes were identified only in children < 2 years old

Table 3.3: Distribution of rotavirus RNA electropherotypes among HIV adults

	Electropherotypes	
Age groups	Long patterns	Short patterns
18-28	2	0
29-38	2	0
39-48	0	1
49-58	1	2
58 and above	2	0

This table shows that long electropherotypes were dominant over short electropherotypes

4.3 Genotyping

Genotyping of VP7 and VP4 was carried out and the results were summarised in table 4.4.

Table 4.4: Prevalence of rotavirus genotypes detected in adults and children.

Genotypes	Children (N=30)(%)	Adults (N=10) (%)	Total (N= (40) (%)
G- types			
G1	14 (47)	0	14(35)
G9	9 (30)	8 (80)	9 (43)
G3	4 (13)	2 (20)	6 (15)
P-types			
P[8]	23 (77)	8 (80)	31(78)
P[6]	2 (6.7)	2 (20)	4 (10)
Mixed infection	2(6.7)	0	2 (5)
Non typeable	3 (10)	0	3(8)

4.3.1 VP7 genotypes

The G types (VP7 associated) were successfully determined in 27 (90%) of the 30 PAGE positive samples. A G type was assigned after PCR with G1, G2, G3, G4, G6, G8 and G9 – specific primers was carried out on rotavirus cDNA/DNA template.

The overall incidence for G typing was G1, 47% (14/30), followed by G9 30% (9), and G3, 13% (4) (Table 4.4). One dual infection was detected at 3.3% (1), while G2, G4 and G8-type viruses were not detected. Two samples, although positive for rotavirus remained untypeable (6.7%). The incidence of each type was seen to vary from month to month. G1 and G9 occurred all year round while G3 occurred during rainy seasons. Figure 4.5 shows an agarose gel displaying various genotypes that were found in circulation in Viwandani slum.

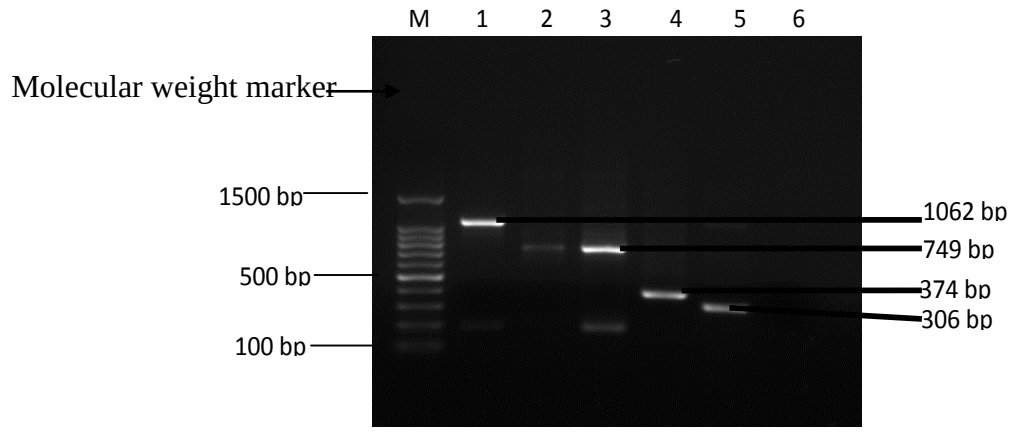

Figure 4.5: Agarose gel under U.V light showing G-genotypes

Lanes are loaded as follows. Lane A contains 100 base pair ladder marker. B contains a full length VP7 gene (1062bp); lane c contains an amplified serotype G1 rotavirus (749bp). Lane D shows non-specific binding; lane E contains an amplified G3 rotavirus (374bp) and lane f contains amplified G9 (306bp).

4.3.2 VP4 genotypes

The P types (VP4 associated) were successfully determined in 25 (83.3%) of the 30 PAGE positive samples (Table 4.5). A P type was assigned after a PCR with P[4], P[6], P[8], P[9] and P[10]- specific primers was carried out on rotavirus cDNA/DNA template.

Only two P types were found to be in circulation in viwandani slums: P[6]- 2 (6.7%) and P[8] 23 (77%). 6.7% P[6]/P[8] dual infections were detected in 2 samples ((Table 4-4), while no P [9] or P[10]-type viruses were detected during the survey. Three samples (10%) were untypeable. The incidence of each type was seen to vary from month to month, with P[8] occurring most frequently in all round the year.

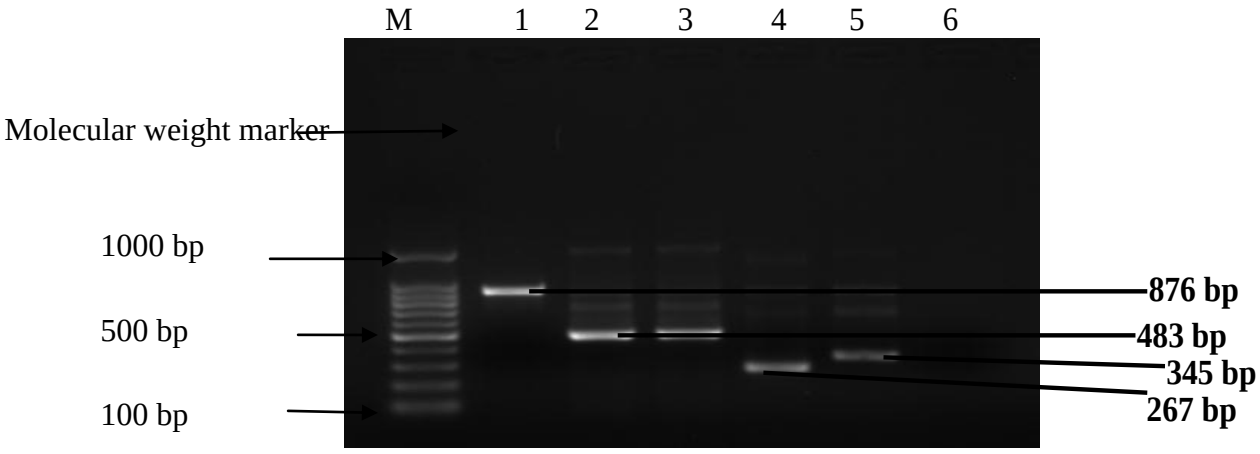

Figure 4.6: Agarose gel under U.V light showing P-genotypes.

Lanes are loaded as follows. Lane M contains 100 base pair ladder marker. 1 contains a full length VP4 gene (876bp); lane 2 and 3 contains an amplified P[4] rotavirus (483bp); lane 4 contains an amplified P[6] rotavirus (267bp) and lane 5 contains amplified P[8] (345bp); 6 negative control.

4.3.3 Combination of G and P types

During the typing assays it was observed that a specific G type could always be correlated to a P type; namely, G1, and G3 always coexisted with P[8], while G9 was found to associate with P[6] or P[4]. The incidence of each genotype varied considerably for the 12 months (table 6). Overall, G1 P[8] was recorded as the most common (44%) in children while G9 recorded the highest percentage in adults 57%. Followed by G3P[8] (24%) in childen. The other types, namely, G9 P[6], G9 P[4], and mixed types (G3/G9 P[8]) were less frequent (20% 12%, and 10% respectively). In children, while in adults G3P[8] followed by 28.6%.

It was noted that G1P[8] was the most predominant type collected from both the male and female children and G9[P6] was the most predominant in adults.

Table 4.5: G and P- type combination detected in children below five year and H.I.V infected adults.

G-P type combination	**Children (N=30)(%)**	**Adults (N=10) (%)**	**Total (N= (40) (%)**
Common types			
G1P[8]	11(37)	0	11(27.5)
G9P[8]	5(16)	4(40)	9(22.5)
G3P[8]	6(20)	3(30)	9(22.5)
Uncommon types			
G9P[6]	3(10)	2(20)	5(12.5)
G1P[4]	0	0	0
Mixed infection	2(7)	0	2(5)
Non typeable			
G* - P**	0	0	0
G** – P*	1(3)	0	1(2.5)
G* - P*	2(7)	1(10)	3(7.5)

*non typeable **positive

4.4 DISCUSSION

This study reports a prevalence of 23% and 8% of rotavirus infection in children below five years and HIV adults respectively. The findings regarding rotavirus prevalence in children reflects previous results which reported rotavirus in 18% in outpatient setting in Meru, Kenya (Kiulia *et al,* 2006) and 30% in an inpatient setting in Kilifi, Kenya (Nokes *et al,* 2008). However a study that was conducted in the Eastern region of Kenya found a prevalence of 38% in two thousand and fourteen (Kiulia *et at*, 2014). Further the rotavirus prevalence finding in this study is similar to findings found in other African countries, where prevalence ranged from 19% in Senegal, 21% in Cote d'Ivoire, to 52% in Ghana (Cunliffe. *et al,* 1998).
Prevalence of HIV positive adults was 8%. These findings were similar to findings of a study done in Lima Peru where the rotavirus prevalence in HIV positive adults was reported to be 8% (Cárcamo *et al,* 2005).

In comparison with a previous study (Basu *et al,* 2003), prevalence of rotavirus diarrhoea was low in infants aged between 1 day old-6 months, peaked 7-12 months and declined among children of 13-16 months of age. The relative low prevalence of viruses among older children could be attributed to acquired immunity through previous infections. Children under 1 year contributed to over 17/30 (57%) of positive cases and over 24/30 (80%) of the children had rotavirus diarrhoea the age of 2 years. The findings of this study were similar to those conducted in Cameroon where it was found out that rotavirus associated diarrhoea occurred early in life, and children below two years of age were the mostly affected (Ndze *et al,* 2011).
There is great variation in occurrence of diarrhoea within children below one year of age from the neonatal stage to the early and late infancy group. Rotavirus infection in newborns is due to mild or asymptomatic disease (Temu *et al,* 2011). It was discovered that few children were infected with rotavirus associated diarrhoea in the first three months of life. This could be due to infants are subjected to exclusive breastfeeding which lowers chances of contamination with rotavirus. During the neonatal stage, infants are protected by passively acquired maternal antibodies. In addition, the inability of neonates to infect themselves through their fingers may

partly explain the lower rate of both diarrhoea and rotavirus at this age group. Neonates on exclusive breastfeeding pass several soft stools per day, so even if they are infected at this young age, the infection can be asymptomatic or can be mild, hence undetected.

The high prevalence of rotavirus caused diarrhoea in the subsequent age groups is explained partly by the waning passive immunity (Temu *et al,* 2011). This early exposure to rotavirus infection does not offer protection against future rotavirus infection as immunity is not acquired at this early age (Moyo *et al,* 2007). Moreover, sthe weaning of infants increases rate of contamination of the gut by pathogens at the same time as the body starts to develop immunity. Hence there is active immunity that protects children against rotavirus infections at the age of one year.

Subsequent rotavirus associated diarrhoea may occur in children 1 year to 5 years can still get diarrhoea from rotavirus and other pathogens, but the infections are less severe, so patients may end up staying at home or visiting the hospital as outpatients. These findings are in agreement with studies done other African countries where rotavirus and other diarrheal pathogens are acquired early in life (Moyo *et al,* 2007).

Similarly a study on generation of rotavirus-specific IgA antibodies following rotavirus infection (Temu *et al,* 2011) found out that, at the age of 11 months, about 85% of the infants acquire rotavirus IgA antibodies. Neutralizing IgG antibodies also increase markedly as the children reached one year of age, reflecting exposure to natural infection and as expected, infection in this age group is generally mild with few deaths reported worldwide.

Further, the rate of rotavirus infection has also been confirmed to increase with age in the first year of life in previous studies. A study conducted in Turkey found out that rotavirus infection affected mainly children 6 to 23 months (Temu *et al,* 2011).

However, a study done in Nigeria showed that the younger the age the higher the risk for rotavirus induced diarrhoea, with the highest burden detected in children less than six months (Temu *et al,* 2011).

This study also noted a low prevalence of rotavirus in older children between 2 to 5 years of age. This could be explained by the acquisition of neutralizing antibodies to rotavirus early in life as a result of multiple exposures to rotavirus infection (Moyo *et*

al, 2007). These findings are important for determining timing of vaccination against rotavirus in different parts of the world (Ramachandran *et al,* 1998)

Rotavirus caused gastroenteritis leads to a high rate of clinic visits and hospital admission related to dehydration in both developing and developed countries (Temu *et al,* 2011). However, children in developing countries die more frequently possibly due to inaccessibility to treatment and high rate of malnutrition (Parashar *et al,* 2003).

Adults above 48 (50%) years of age were susceptible to rotavirus infection and this could be explained by the weakened immunity in older subjects and the underlying immune-suppression particularly in those with HIV infection (Cárcamo *et al,* 2005).

There was no significant difference between this study and a study done in China on distribution of rotavirus infection between male and female gender in children *p=0.8350* (Qiao *et al,* 1999). Despite the low number of adults which was insufficient to make any meaningful comparison there was also no statistical difference observed in HIV adults based on gender *p*=1.00. Nevertheless, when the sample size was expressed as percentage of proportion, (70%) were women and (30%) were men. Studies on adult rotavirus diarrhoea and their influence on children rotavirus infection have not been conducted (Shen *et al,* 2013). However, a study carried out by (Anderson and Weber, 2004) described that adults in close contact with children particularly have high chances of acquiring rotavirus infection, rates going from 33% to 55% of adults that care for children at home. This findings were also confirmed by (Grimwood *et al,* 1983) who found out that, the major primary mechanism that aided spread of rotavirus infection was intra-familial contacts. In addition, he also demonstrated that, there was a high likelihood of adults in close contact with rotavirus infected children developing rotavirus infection. These adults should be identified with an isolation procedure for preventing this condition as well as prevent antibiotic misuse (Anderson and Weber, 2004). Therefore, transmission of rotavirus infection within families from children to parents seems to be a common event. The high percentage of women 70% that were rotavirus positive were probably as a result of the close contact with children since they are the women who spend a lot of time taking care of children.

There was significant difference observed between children and H.I.V adults based on distribution of rotavirus infection *p=0.0002*. This could be a result of rotavirus infections are mainly known to affect children less five years old (Ramachandran *et al,* 1998) while rotavirus infections in adults are rare but common in immune-insufficient adults (Anderson and Weber, 2004).

This study found out that rotavirus was in circulation throughout the year. Rotavirus infection in both adults and children revealed a marked seasonal variation with incidence being high during wet and cold months of March April and November and dry month of August. These finding was consistent to those conducted in South Africa (Cunliffe *et al,* 2006) and Botswana by (Basu *et al,* 2003). However, an overview done by (Midthun, 1996) found out there was no seasonal pattern of rotavirus infection observed in tropical countries. It has been proved that rotavirus infections frequently occur during the rainy season in tropical climates (Labaron *et al,* 1990). This is attributed to the fact that, the rainy season facilitates conditions which increase the rate of transmission such a poor sanitation and poor drainage system in developing nations (Cunliffe *et al,* 1998). This is in agreement to the fact that rotavirus infection is highly infectious and spreads through fecal-oral route although; respiratory transmission may also occur (Midthun, 1996). In addition, rotavirus is hard to be eliminated through improvement in water and sanitation moreover the low infectious dose (10–100 virus particles), makes it hard to stop transmission of rotavirus although, if good hygiene measures are kept in place they can help to prevent the transmission of rotavirus caused gastroenteritis (Steele and Glass, 2011b). Therefore development of a safe and effective rotavirus vaccine is necessary. In conclusion the poor drainage system and poor sanitation in viwandani slum explains why rotavirus infection is present in all seasons.

Electropherotypes detected by PAGE showed great similarity in both children and adults. Long electropherotypes were dominant 24/30 (80%) while the short electropherotypes were detected in 6/30 (20%) samples in children while long electropherotypes were also dominant in H.I.V adults accounting for 60% while short electropherotypes accounted for 40%. This is in support of a review carried out

by (Cunliffe *et al,* 1998; Kiulia *et al,* 2006). Long strains occurred throughout all seasons of the year while the short strains were found during the cold season. These results agree with a study carried out in South Africa (Banyai *et al,* 2002). Long RNA electropherotypes were distributed in all age groups (one month to five years old), while the short RNA profiles were only identified in children two years and below.

The monthly distribution of the long electropherotypes appeared to be random and no apparent seasonal variations could be detected. The short profiles were more prevalent during cold and dry months and were not observed in dry months of the study period (Table 4.2 and 4.3).

Genotyping is an epidemiological tool used in examining diversity of rotavirus strains, and the combination of P and G genotypes. Molecular methods like RT-PCR with increased sensitivity allow characterization of rotavirus VP7 and VP4 accurately (Gentsch *et al,* 2005).

The majority of the serotypes in circulation in viwandani slum were similar to those that have been found globally (Gentsch *et al,* 2005). Only three G-serotypes G1, G9 and G3 were found to be in circulation in Viwandani slum. Consistently with findings of numerous studies done, G1 was the most predominant serotype (47%) detected in children in viwandani slum, Nairobi, G9 (30%) was the second and G3 was the third in prevalence. However, a study conducted by kiulia in Eastern region of Kenya showed that G9 was the most predominant G-serotype accounting for 51%. Additionally, he detected G12 genotype for the first time in Kenya that accounted for 3.1% (Kiulia *et al,* 2014). (Kiulia *et al,* 2006), also showed that G9 (47%) was the most predominant serotype followed by G8 (29%) and G1 (4%) respectively.

Further, this findings were similar to findings of a study done in India that found G1 (47%) the most prevalent genotype causing rotavirus associated gastroenteritis in children (Kang *et al,* 2005). On the contrary, studies have shown that G9 genotype is the prevalent cause of rotavirus infection. Studies done in Thailand (Jiraphongsa *et al,* 2005) and china (Shen *et al,* 2013) indicated that G9 viruses were dominant causative agent of rotavirus caused gastroenteritis in children.

This suggests the recurring changing genotypes of rotavirus circulating in Kenya with G1 and G9 being the most predominant. In addition, this could indicate emergence of new rotavirus strains or inadequate diagnosis of rotavirus serotypes in the past. Therefore, there is need of continuous surveillance of rotavirus strains if the present vaccines will assist in conferring immunity against rotavirus caused gastroenteritis.

Although, it was difficult to make accurate conclusion on genotype diversity due to low number of samples recruited in this study. Two genotypes were found in circulation in Viwandani slum affecting H.I.V infected adults. G9 (80%) was predominant followed by G3 (20%). These findings were in comparison to previous studies done in Sweden (Rubilar-abreu *et al,* 2005). However, other studies conducted in china found out that G1 viruses were the dominant type causing gastroenteritis in H.I.V infected adults.

Samples having been collected during the same period and from the same geographical area were an indication of symptomatic G9 infections in both the adults and children demonstrating that these infections occurred independently of the pre-existing immunity. This could be due to infections from rotavirus don't confer lifelong immunity further exposing them to repeated rotavirus infections that vary from asymptomatic to severe depending on the immunity of the host (Temu *et al,* 2011).

Therefore asymptomatic adults infected with rotavirus could be a source of maintaining rotavirus in circulation in the population as well as a reservoir of genetic diversity that may limit the effectiveness of rotavirus vaccine used in children. Although G9 has not been included in current vaccines, data from previous studies has suggested inclusion of cross protection of vaccines against G9 (Shen *et al*, 2013). However the high prevalence of G9 should be interpreted with a lot of caution due to insufficient data on vaccine evaluation in Viwandani slum. Therefore it is of great significance to continue with surveillance of rotavirus strains in this region.

P-typing was done and P [8] serotype was the most prevalent (59%) that was found in 17 cases followed by P[6] (19%), P4 (10%) respectively in children.

The findings in this study together with those conducted by (Gentsch *et al,* 2005) found out that P[6] protein that was traditionally thought to be associated with asymptomatic neonatal disease is also identified in older chilldren. Suggesting that reassortment in neonates could be one possible source for new strains. The incidence of P[6] in recent American (Ramachandran *et al,* 1998), Brazilian (Timetesky *et al,* 1994), and Indian (Ramachandran *et al,* 1996) studies highlight this strain as an emerging genotype.

Five samples only, could not be successfully assigned a G or a P type indicating that the emergency of unusual strains in viwandani slum is rare. Compared to other other studies, in which approximately 30% of rotavirus positive stool specimens could not be assigned a P or G typed (Cunliffe *et al,* 1998),

The low efficiency relative to P typing can be attributed to the degradation of the nucleic acid from some of the earlier isolates, since the G-typing assays were completed first. Lack of sufficient sample to carry out p-typing could also contribute to low efficiency in P-typing.

Molecular typing methodology has enhanced typing knowledge of G and P genotype diversity (Gouvea *et al,* 1990)**.** G and P type combinations were classified into common and uncommon categories as described by (Tcheremenskaia *et al,* 2007) as shown in table 4-5. G-type could be identified in only 90% of the specimen and P-type in only 83% of the specimen. Analysis of individual specimens indicated that 83% had a combination of G and P types, therefore reflecting a single rotavirus strain and 6.7 % had neither a G-type nor a P-type identified. In comparison with previous studies, genotypes G1 and G3 were found to correlate with serotype P[8] while G9 was found to correlate with serotype P[6] and P[8] (Ramachandran *et al,* 1998). G1[P8] (40%) was the most in children which is the common combination in most parts of the world (Ramachandran *et al,* 1998) whereas G9[P8] (80%)] was the most common in adults. The rarely seen G9P[6] combinations identified highlight the possibility of of rotaviruses to undergo re-assortment (Gentsch *et al,* 2005). Though this study was conducted in health two health centres in Viwandani slum correlation did not exist between the hospital source and a particular genotype. Genotypes were evenly distributed in all collection centres in viwandani slums. The detection of

rotavirus genotypes for the first time in children from viwandani slums is an indication that the prevalence of certain genotypes may change with time in this locality. Hence this study is significant as it mirrors observations from studies in other developing countries (Cunliffe. *et al,* 1998).

Only two samples (6.7%) could not be assigned a G or a P type, which suggests that the incidence of unusual genotypes in Viwandani is rare. This figure is low when compared to the data from other studies, in which approximately 30% of rotavirus positive stool specimens could not be P or G typed. The occurrence of untypeable strains in this study is unclear. There are many factors that can contribute to detection of untypeable strains. Presence of inhibitors during extraction of RNA and primer binding region could inhibit genotyping. Most sequencing studies indicate that these untypeable serotypes belong to the common four genotypes (Ramachandran *et al,* 1998) however, a study that was done in brazil, demonstrated the existence of unusual genotypic diversity and complexity among the strains recovered (Santos and Gouveas, 1994). This justifies the fact that the presence of untypeable strains could be an indication of emergency of new strains that can only be confirmed by sequencing. This is an indicator that the effectiveness of vaccines may be limited against this unknown antigenic challenge (Cunliffe *et al,* 1998)**.** Therefore further research by sequencing should be considered in future.

Similarly to a study done by (Gentsch *et al,* 2005) three samples (10%) had mixed infections of G1 and G4 and G3 and G9 . Mixed infections are frequent in developing countries. These findings were in supportive of a study done by (Richardson *et al,* 1998) that found dual infections with serotypes G1 and G4 in single individual reported among the children. A review done in Africa by (Cunliffe *et al,* 1998) found out that children who suffered from rotavirus infection were associated to malnutrition and prolonged diarrhoea (Richardson *et al*, 1998). Therefore these mixed infections could be a result of high levels of poverty in Viwandani slums where malnutrition and prolonged diarrhoea are expected.

This study found out G1/G4 and G3/G9 mixed infection in a single stool specimen. Hence mixed infection could be a result of multiple microbial infection in subjects i.e. more than one pathogen in a single patient (Steele & Glass, 2011a).

CHAPTER FIVE

5.1 Introduction

This chapter will provide a brief summary of the findings of this study and highlight the importance of my results to the vaccine design that is faced with challenges of rapid antigenic changes due to viral reassortment, natural occurring mutations and hence diverse viral strains.

5.2 Summary

Rotavirus is a leading cause of severe diarrhoea in children in Viwandani slum where a prevalence of 23% was established in this study. It is therefore important to document the local strain(s) of rotavirus in circulation in various regions to ensure the success of rotavirus vaccine. However, rotavirus was not found to be a major cause of severe gastroenteritis in HIV adults s since a prevalence of 8% was established.

This study was aimed at characterizing human rotavirus group A serotypes causing gastroenteritis among children below five years of age and HIV-infected adults in Viwandani slum, Nairobi. A cross-sectional, hospital based study was conducted and a total of 260 faecal specimen samples were collected for analysis (128 from children and 132 from HIV infected adults) during the period between August 2012- July 2013 from Mukuru kwa Njenga and Mukuru kwa Reuben health centres in viwandani slums in Nairobi.

The samples were detected for rotavirus strains using antigen based enzyme immune-sorbent assay (ELISA), Polyacrylamide Gel Electrophoresis and Real Time Polymerase Chain Reaction to detect rotavirus infection in faecal samples. ELISA was used to detect rotavirus prevalence, Polyacrylamide gel electrophoresis was used to detect rotavirus electropherotypes and finally genotyping was done by RT-PCR to confirm rotavirus genotypes using genotype-specific primer sets targeting VP4 and VP7 genes.

This study found out that rotavirus was in circulation throughout the year however**,** with high incidence cases being detected during wet and cold months of March April

and November and dry month of August. Prevalence was high among children aged two yrs and adults above age of 48 yrs. The common globally distributed strains, G1, G2, G3 and G4, accounted for 60% detections while G9 strain accounted for 80% infection in adults. G1[P8] was the common genotypic combination in children, accounting for 40% infection, whereas G9[P8] accounted for 60% of the infection in adults.

This study concludes that there is strain diversity in rotavirus circulating in Viwandani slums in Nairobi. In addition, the study asserts that the two rotavirus vaccines recommended for world, cover all the circulating in Viandani. It is recommended that molecular epidemiology of rotavirus especially in low income settlement be a continuous process especially among HIV infected.

5.3 Conclusions

Rotavirus caused gastroenteritis is the major cause of severe dehydrating diarrhoea in children in Viwandani slum. This study found out that children below 2 yrs of age that accounted for 80% of the sample size were most affected. Adults who were 48 yrs of age and above were also highly susceptible to rotavirus infections however rotavirus was not found to be a major cause of severe diarrhoea in HIV positive adults in this study.

Long electropherotypes were predominant to short electropherotypes in both children below five years and HIV positive adults accounting for 8o% and 60% respectively.

Finally G1 was the main serotypes affecting children while G9 was the most prevalent serotype in circulation affecting adults.

Epidemiologic surveillance of rotavirus is key step in understanding the burden of rotavirus infection in human health and their impact on national health cost in Kenya. To date no epidemiologic surveillance of acute gastroenteritis caused by rotavirus has been established in Kenya. Therefore the government should be encouraged to carry out surveillance studies in order to document these strains. This important information will help in improving the health system needed in Kenya and it will also form a platform of addressing specific questions of relevance to the global community including safety of rotavirus vaccines in HIV infected children.

5.4 Recommendations

1. Rotavirus surveillance program should be continuously conducted to support tracking most recent strains in vaccine design.
2. Simple, inexpensive rapid tests for rotavirus detection should be encouraged in local health centres and this can be beneficial on the other hand since it can be a basis of national surveillance system.
3. Co factors which may influence severity of rotavirus disease e.g. H.I.V, malaria and other enteric pathogens should be put into account and there of investigated on. In addition correlation of viruses with clinical symptom severity should be performed.
4. Finally, we only investigated few strains of rotavirus. All common strains of this virus should be investigated in future studies. Moreover only patients presenting to a hospital were included in this study. To investigate the true burden of viral gastroenteritis, community-based studies are necessary.

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APPENDICES

Appendix i Informed Consent Form

Consent form Explanation in English

ADULTS CONSENT FORM (ADULTS/ SUBJECT)

TITLE: Characterization of human rotavirus strains causing gastroenteritis among children less five years and H.I.V adults in Viwandani slum, Nairobi.

INVESTIGATORS

PRINCIPAL INVESTIGATOR

1. Raini Sandra Kendra, Msc (Med Virology) Hons-ITROMID, KEMRI

RESEARCH SUPERVISORS

1. Dr.Janeth kombic - KEMRI
2. Dr.Juliette Ongus - JKUAT

INTRODUCTION

My name is _____. I wish to introduce you to this study undertaken by Raini Sandra Kendra, a Masters student at Jomo Kenyatta University of Agriculture and Technology's Institute of Tropical Medicine and Infectious Diseases- KEMRI. I would be very grateful if you would volunteer to participate in this study.

WHY IS THE STUDY BEING DONE?

This research looks at the type of a virus known as `rotavirus` causing diarrhoea in children less 5 years. It also looks at the possibility of the virus and the type that causes diarrhoea in H.I.V positive adults. Our scope is mainly in Viwandani slums, Nairobi. The results of this study will be given to the Kenya Medical Research Institute (KEMRI) who will take the necessary action depending on the outcomes. The study will have a long term benefit to the general population as the results will provide information on circulating strains in the region and highlight the importance of rotavirus in infected adults.

HOW MANY PEOPLE PARTICIPATE IN THE STUDY?

A total of 260 (128 children and 132 adults are expected to participate in the study.

WHAT IS INVOLVED IN THE STUDY?

The study involves random collection of stool samples from out patients attending Mukuru kwa njenga missionary hospital. H.I.V adults, samples will be collected from out patients who are already detected positive according to W.H.O standards still attending the clinic. You will be provided with a sterile container within which to collect the stool during the clinic visit. Ward patients will be given containers in which stools will be collected in the morning. Samples will be collected and transported to KEMRI for analysis

CONSENT FOR STORAGE AND TRANSPORTATION OF SAMPLES FROM MUKURU KWA NJENGA MISSIONARY HOSPITAL TO CENTRE FOR VIRUS RESEARCH (KEMRI)

YES ☐ NO ☐

PLEASE CHECK ONE BOX ONLY

HOW LONG WILL THE SAMPLE COLLECTION TAKE?

It is projected to take 3 months

WHAT ARE THE RISKS OF THE STUDY?

You will collect the stools in the containers in the normal defecation process. This will therefore not subject you to any pain.

ARE THERE BENEFITS TO TAKING PART IN THE STUDY

Subjects will not have any direct benefits however, the results of the study will be shared with KEMRI and other relevant authorities, who will take measures to advice the general public, based on the results, and take the necessary course(s) of action. As already stated, results obtained will provide information on the circulating strains in the region and highlight the importance of rotavirus in HIV adults

WHAT ABOUT CONFIDENTIALITY?

All the information obtained will be strictly confidential and data will be password protected. Only the Principal investigator, the research supervisors, officials in Mukuru kwa njenga missionary hospital and if need be, appointed authority from the KEMRI/ National Ethical Review Committee will be able to access the information. You will remain anonymous during and after the study, being identified only by the Personal Identification Numbers (PIN) assigned to you. The results obtained will be made available to the health care givers only with your consent.

WHAT ARE THE COSTS?

You do not pay anything to participate in this study.

SUMMARY OF YOUR RIGHTS AS A PARTICIPANT IN A RESEARCH STUDY

This study is absolutely voluntary, you will be free to withdraw from the study at any point and will not be penalized in any way, and, you are not also waiving any of your legal rights by signing this informed consent document.

CONTACT INFORMATION

The following person will be available for contact in the event of any research related questions or comments.

Principal Investigator: Raini Sandra Kendra, JKUAT.

Telephone: 0726-379359

Email: kendraraini@yahoo.com

And in case of complaints regarding rights of participation, one can always contact

KEMRI/NERC: THE SECRETARY

Telephone: 2722541 Ext. 3307

Mobile: 0722205901 / 073340003

YOUR PERMISSION

I have read the Consent Form and conditions of this project. All my questions have been answered and I hereby give my voluntary consent.

CONSENT FOR STOOL COLLECTION GIVEN YES ☐ NO ☐

SIGNATURE OF PARTICIPANT _____ DATE _____

PRINTED NAME OF PARTICIPANT _____

Name and signature of the witness_____ sign _____

* Only in case the subject is not literate and a thumb is used. *Must not be the person collecting specimen

SIGNATURE OF PERSON OBTAINING CONSENT

_____ DATE _____

DATE PRINTED NAME OF PERSON OBTAINING CONSENT

(Must be the principal investigator or individual who has been designated in the checklist to obtain consent)

SIGNATURE OF THE PRINCIPAL INVESTIGSTOR

DATE

_____ _____

Appendix ii Consent Form Explanation in Kiswahili

KARATASI YA KUFAHAMISHA IDHINI (WATU WAZIMA)

KICHWA: Uchunguzi wa aina ya virusi vya rota vinavyosababisha ugonjwa wa kuhara katika maeneo ya viwandani,kitongoji duni, Nairobi.

WACHUNGUZI:

MCHUNGUZI MKUU

1. Name Raini Sandra Kendra

WASAIDIZI

2. Dr. Janeth Kombich, PhD- KEMRI
3. Dr Juliette ongus

UTANGULIZI

Jina langu ni _____. Ninakualika ushiriki katika uchunguzi huu unaofanywa na Raini Sandra Kendra, mwanafunzi wa chuo kikuu cha Jomo Kenyatta. Lengo kuu ya kazi hii ni kuchunguza aina ya virusi vya rota vinavyosababisha ugonjwa wa kuhara katika maeneo ya viwandani,kitongoji duni, Nairobi. matokeo yatafaidi jamii kwa jumla kwa kutujulisha aina ya virusi ambavyo viko maeneo ya viwandani, Nairobi. Nitashukuru sana indhini yako ya kushiriki katika mradi huu.

MADHUMUNI

Mradi huu unachunguza aina ya virusi vya rota vinavyosababisha ugonjwa wa kuhara katika maeneo ya viwandani,kitongoji duni, Nairobi. Sampuli sitakusanywa kutoka hospital ya mukuru kwa njenga kutoka kwa wagonjwa ambao wanakuja sahanatini kupata matibabu.Matokeo ya uchunguzi huu yatawasilishwa kwa Kituo cha Utafiti ya magonjwa mbalimbali ya binadamu (Kenya Medical Research Institute- KEMRI), kitakachochukua jukumu kufuatiana na matokeo ya utafiti huu kuelimisha na kusaidia uma kwa njia mbalimbali. Matokeo ya kazi hii pia yatafaidi jamii kwa jumla kwa kutujulisha aina ya virusi vya rota katika maeneo ya viwandani, kitongoji duni Nairobi.

IDADI YA WASHIRIKI

Utafiti huu utahusisha washiriki 128 ambao watakuwa ni watoto wachanga wasiozidi umri wa miaka mitano na 132 watakuwa ni watu wazima wasiopungua miaka kumi na nane walioambukizwa ugonjwa wa ukimwi.

TARATIBU ZA UTAFITI

Uchunguzi unafanywa kwa wagonjwa wanaopata matibabu katika hospitali ya mukuru kwa njenga. Unahusisha uchunguzi wa vinyesi vinavyotolewa na wangojwa wanaohisi maumivu ya tumbo yanayosababisha ugonjwa wa kuhara. Utapewa chupa utakaotumia kuweka sehemu ya kinyesi na kurudisha hospitalini. Wagonjwa wanaolazwa watahitajika kutoa vinyesi asubuhi na kuletwa zahanatini. Vinyesi vitakusanywa na kuhifadhiwa. Baadaye sampuli hizi zitasafirishwa hadi chuo cha utafiti cha KEMRI, nchini , kufanyiwa utafiti ya ziada.

IDHINI YA KUHIFADHI NA KUSAFIRISHA SAMPULI KUTOKA HOSPITA LI YA MUKURU KWA NJENGA HADI CHUO CHA UTAFITI CHA KEMRI

NDIO ☐ LA ☐

CHAGUA MOJA

NI MUDA GANI NITADUMU KWA MRADI?

Mradi huu utachukua muda wa miezi tatu

MADHARA

Hakuna uchungu wowote utakaohisi, utaweka kinyesi ndani ya chupa wakati wa kuenda haja kubwa kama kawaida.

FAIDA

Hakuna faida yoyote ya kibinafsi utapata. Ya muhimu ni kwamba majibu ya utafiti huu yatatumiwa, baada ya idhini ya mshiriki, kuelimisha na kusaida uma. Matokeo ya kazi hii pia yatafaidi jamii kwa jumla kwa kutujulisha aina ya virusi vya rota katika maeneo ya viwandani, Nairobi.

SIRI YA HALI YAKO

Matokeo ya utafiti huu yatawekwa kwa siri kuu. Mchunguzi mkuu, wasaidizi wa uchunguzi ,maofisa ambao wanahusika kutoka hosipitali ya viwandani na kutakapokuwa na haja, atakayeidhinishwa na KEMRI/NERC ndio pekee watakua na

idhini ya kuyafikia. Hakuna jina litakalochapishwa popote wakati hata baada ya uchunguzi kukamilika. Utajulikana kwa nambari ya siri utakaopewa baada ya idhini yako ya kushiriki.

GHARAMA

Hakuna ada au gharama yoyote utatozwa ili kushiriki katika mradi huu.

MUHKTASARI JUU YA HAKI ZAKO KAMA MSHIRIKI KATIKA MRADI

Kushiriki kwako katika mradi huu ni kwa hiari yako na unaweza kujiondoa wakati wowote bila kupoteza faida yeyote inayotokana na mradi huu. Vilevile hutahujumu haki zako kwa kutia kidole kwenye stakabali hii.

HABARI ZINAPATIKANA

Maswali yoyote kuhusu mradi huu yanastahili kuelekezwa kwa

Mchunguzi mkuu: Raini Sandra Kendra- Bsc.(Biochemistry) K.U.
Simu: 0726-379359.
Pepe: kendraraini@yahoo.com

Pia maswali kuhusu haki zenyu kuhusu kuchangia kwenye mradi huu yanaweza kuelekezwa kwa

KEMRI/NERC: Kiranja
Nambari ya simu: 2722541 Ext. 3307
Simu ya mkono: 0722205901 / 073340003

IDHINI YA MUHUSIKA

Nimeisoma fomu hii na masharti ya mradi huu. Maswali yangu yote yamejibiwa na kwa hivyo ninatoa idhini ya sampuli zangu kutumiwa kwa mradi huu.

IDHINI YA KUCHUKUA KINYESI NDIO ☐ LA ☐

CHAGUA MOJA

Sahihi ya mshiriki_____Tarehe_____

Jina_____ Tarehe_____

Jina la Shahidi_____ Sahihi_____

*Kwa wale washiriki wasiojua kusoma na kuandika * Lazima awe mtu huru

Sahihi ya anayechukua idhini_____Tarehe_____

Jina_____Tarehe_____

Lazima awe mtafiti/mchunguzi ama mtu aliyepewa jukumu la kupewa idhini)

Sahihi ya mchunguzi mkuu

_____Tarehe_____ (Anayethibitisha kuwa mshiriki anafaa kushiriki na kwamba karatasi ya idhini imepatikana)

Appendix iii Consent form Explanation in Kiswahili for Children

ADULTS CONSENT FORM (PARENT/GUARDIAN)

Title: Characterization of human rotavirus strains causing gastroenteritis among children less 5 yrs and HIV positive adults in viwandani slum, Nairobi.

INVESTIGATORS

PRINCIPAL INVESTIGATOR

1. Raini Sandra kendra

RESEARCH SUPERVISORS

2. Dr. Janet kombich, PhD- KEMRI
3. Juliette Ongus

INTRODUCTION

My name is _____. I wish to introduce you to this study undertaken by Raini Sandra Kendra, a Masters student at Jomo Kenyatta University of Agriculture and Technology's Institute of Tropical Medicine and Infectious Diseases- KEMRI. I would be very grateful if you would volunteer to participate in this study.

WHY IS THE STUDY BEING DONE?

This research looks at the type of a virus known as `rotavirus` causing diarrhoea in children below 5 years. It also looks at the possibility of the virus and the type that causes diarrhoea in H.I.V positive adults. Our scope is mainly centred in viwandani,slum, Nairobi. The results of this study will be given to the Kenya Medical Research Institute (KEMRI) who will take the necessary action depending on the outcomes. The study will have a long term benefit to the general population as the results will provide information on the circulating strains in the region and highlight the importance of rotavirus in HIV adults.

HOW MANY PEOPLE PARTICIPATE IN THE STUDY?

128 children less than 5 yrs and 132 HIV antibody positive adults are expected to participate in the study.

WHAT IS INVOLVED IN THE STUDY?

The study involves random collection of stool samples from out patients attending Mukuru kwa njenga missionary hospital. HIV patient's samples will be collected from those who are already diagnosed positive according to W.H.O standard still attending the clinic . Included in the study are stool samples collected from HIV positive adults (≥18years), willing to participate. Your child will be provided with a sterile container within which to collect the stool during the clinic visit. Ward patients will also be given containers in which stools will be collected in the morning. Samples will be collected and transported to KEMRI for typing.

CONSENT FOR STORAGE AND TRANSPORTATION OF SAMPLES FROM MUKURU KWA NJENGA MISSIONARY HOSPITAL TO CENTRE FOR VIRUS RESEARCH KEMRI

YES ☐ NO ☐

PLEASE CHECK ONE BOX ONLY

HOW LONG WILL THE SAMPLE COLLECTION TAKE

It is projected to take 3 months

WHAT ARE THE RISKS OF THE STUDY?

This study does not involve any invasive procedure. You will collect the stools in the containers in the normal defecation process. This will therefore not subject you to any pain.

ARE THERE BENEFITS TO TAKING PART IN THE STUDY?

Subjects will not have any direct benefits however, the results of the study will be shared with KEMRI and other relevant authorities, who will take measures to advice the general public, based on the results, and take the necessary course(s) of action. As already stated results obtained will provide information on circulating strains in the region and highlight the importance of rotavirus in HIV infected adults.

WHAT ABOUT CONFIDENTIALITY?

All the information obtained will be strictly confidential and data will be password protected. Only the Principal investigator, the research supervisors, relevant officials at mukuru kwa njenga hospital and if need be, appointed authority from the KEMRI/ National Ethical Review Committee will be able to access the information. Your child will remain anonymous during and after the study, being identified only by the Personal Identification Numbers (PIN) assigned to you. The results obtained will be made available to the health care givers only with your consent.

WHAT ARE THE COSTS?

You do not pay anything for your child to participate in this study.

SUMMARY OF YOUR CHILD'S RIGHTS AS A PARTICIPANT IN THE STUDY

This study is absolutely voluntary, your child will be free to withdraw from the study at any point and will not be penalized in any way, and, your child is also not waiving any of his/her legal rights by you signing this informed consent document.

CONTACT INFORMATION

The following person will be available for contact in the event of any research related questions or comments.

Principal Investigator: Raini Sandra Kendra- Bsc.(Biochemistry) Hons, Kenyatta university.

Telephone: 0726-379359.

Email: Kendraraini@yahoo.com

And in case of complaints regarding rights in participation of the study, one can contact

KEMRI/NERC: THE SECRETARY

Telephone: 2722541 Ext. 3307

Mobile: 0722205901 / 073340003

YOUR PERMISSION

I have read the Consent Form and conditions of this project. All my questions have been answered and on behalf of _____, I hereby give my voluntary consent.

CONSENT FOR STOOL COLLECTION GIVEN YES NO

PLEASE CHECK ONE BOX ONLY

SIGNATURE OF PARTICIPANT _____ DATE _____

PRINTED NAME OF PARTICIPANT _____

Name and signature of the witness _____ sign _____

* Only in case the subject is not literate and a thumb is used. *Must not be the person collecting specimen

SIGNATURE OF PERSON OBTAINING CONSENT DATE

PRINTED NAME OF PERSON OBTAINING CONSENT _____ _____

(Must be the principal investigator or individual who has been designated in the checklist to obtain consent)

SIGNATUREOF PRINCIPAL INVESTIGATOR

DATE _____ _____

Appendix iv Consent Form Explanation In Kiswahili For Children

KARATASI YA KUFAHAMISHA IDHINI (MZAZI/MSIMAMIZI)

KICHWA: Uchunguzi wa aina ya virusi vya rota vinavyosababisha ugonjwa wa kuhara katika maeneo ya viwandani, kitongoji dunu,Nairobi.

WACHUNGUZI:

MCHUNGUZI MKUU

Raini Sandra Kendra

WASAIDIZI

Dr. Janeth kombich, PhD- KEMRI

Dr Julliette Ongus, PhD-JKUAT

UTANGULIZI

Jina langu ni _____. Ninakualika ushiriki katika uchunguzi huu unaofanywa na Raini Sandra Kendra, mwanafunzi wa chuo kikuu cha Jomo Kenyatta. Lengo kuu ya kazi hii ni kuchunguza aina ya virusi vya rota vinavyosababisha ugonjwa wa kuhara katika sehemu ya Viwandani. matokeo yatafaidi jamii kwa jumla kwa kutujulisha kuhusu aina ya virusi vya rota ambavyo viko sehemu ya viwandani,Nairobi. Nitashukuru sana indhini yako ya kushiriki katika mradi huu.

MADHUMUNI

Mradi huu unachunguza aina ya virusi vya rota vinavyosababisha ugonjwa wa kuhara katika maeneo ya viwandani, kitongoji duni, Nairobi. Matokeo ya uchunguzi huu yatawasilishwa kwa Kituo cha Utafiti ya magonjwa mbalimbali ya binadamu (Kenya Medical Research Institute- KEMRI), kitakachochukua jukumu kufuatiana na matokeo ya utafiti huu kuelimisha na kusaidia uma kwa njia mbalimbali. Matokeo ya kazi hii pia yatafaidi jamii kwa jumla kwa kutujulisha kuhusu aina ya virusi vya rota ambavyo viko maeneo ya viwandani,Nairobi.

IDADI YA WASHIRIKI

Utafiti huu utahusisha washiriki 128 ambao ni watoto wachanga wasiozidi miaka mitano na 132 ambao ni watu wazima ambao wana ugunjwa wa ukimwi

TARATIBU ZA UTAFITI

Uchunguzi unafanywa kwa wagonjwa wanaopata matibabu katika hospitali ya Mukuru kwa njenga,kitongoji duni, Nairobi. Unahusisha uchunguzi wa vinyesi vinavyotolewa na wangojwa wanaohisi maumivu ya tumbo yanayosababisha ugonjwa wa kuhara. Utapewa chupa utakaotumia kuweka sehemu ya kinyesi na kurudisha hospitalini. Wagonjwa wanaolazwa watahitajika kutoa vinyesi asubuhi na kuletwa zahanatini. Vinyesi vyenye virusi vya rota baadaye vitasafirishwa hadi KEMRI, kufanyiwa utafiti ya ziada.

IDHINI YA KUHIFADHI NA KUSAFIRISHA SAMPULI KUTOKA HOPITALI YA MUKURU KWA NJENGA HADI CHUO CHA UTAFITI KEMRI

NDIO ☐ LA ☐

NI MUDA GANI NITADUMU KWA MRADI?

Mradi huu utachukua muda wa miezi tatu

MADHARA

Hakuna uchungu wowote utakaohisi, utaweka kinyesi ndani ya chupa wakati wa kuenda haja kubwa kama kawaida.

FAIDA

Hakuna faida yoyote ya kibinafsi utapata. Ya muhimu ni kwamba majibu ya utafiti huu yatatumiwa, baada ya idhini ya mshiriki, kuelimisha na kusaida uma. Matokeo ya kazi hii pia yatafaidi jamii kwa jumla kwa kutujulisha kuhusu aina ya virusi vya rota ambavyo viko katika maeneo ya viwandani,Nairobi.

SIRI YA HALI YAKO

Matokeo ya utafiti huu yatawekwa kwa siri kuu. Mchunguzi mkuu, wasaidizi wa uchunguzi na kutakapokuwa,maofisa ambao watahusika katika hosipitali ya mukuru kwa njengai na haja, atakayeidhinishwa na KEMRI/NERC ndio pekee watakua na idhini ya kuyafikia. Hakuna jina litakalochapishwa popote wakati hata baada ya uchunguzi kukamilika. Utajulikana kwa nambari ya siri utakaopewa baada ya idhini yako ya kushiriki.

GHARAMA

Hakuna ada au gharama yoyote utatozwa au mtoto wako ili kushiriki katika mradi huu.

MUHKTASARI JUU YA HAKI ZAKO KAMA MSHIRIKI KATIKA MRADI

Kushiriki kwako katika mradi huu ni kwa hiari yako na unaweza kujiondoa wakati wowote bila kupoteza faida yeyote inayotokana na mradi huu. Vilevile hutahujumu haki zako kwa kutia kidole kwenye stakabali hii.

HABARI ZINAPATIKANA

Maswali yoyote kuhusu mradi huu yanastahili kuelekezwa kwa

Mchunguzi mkuu: Raini Kendra Sandra, JKUAT.
Simu: 0726-379359
Pepe: Kendraraini@yahoo.com

Pia maswali kuhusu haki za kuchangia kwa mradi huu zinawezwa elekezwa kwa

KEMRI/NERC: Kiranja
Nambari ya simu: 2722541 Ext. 3307
Simu ya mkono: 0722205901 / 073340003

IDHINI YA MUHUSIKA

Nimeisoma fomu hii na masharti ya mradi huu. Maswali yangu/ mtoto yote yamejibiwa na kwa hivyo kwa niaba ya_____ ninatoa idhini ya sampuli zangu kutumiwa kwa mradi huu.

IDHINI YA KUCHUKUA KINYESI *CHAGUAMOJA*

NDIO ☐ LA ☐

SAHIHI YA MSHIRIKI

_____Tarehe_____

Jina_____ Tarehe_____

Jina la shahidi _____ Sahihi_____

*Kwa wale washiriki wasiojua kusoma na kuandika * Lazima awe mtu huru

Sahihi ya anayechukua idhini_____ Tarehe_____

Jina_____Tarehe_____

Lazima awe mtafiti/mchunguzi ama mtu aliyepewa jukumu la kupewa idhini)

Sahihi ya mchunguzi mkuu

_____Tarehe_____

(Anayethibitisha kuwa mshiriki anafaa kushiriki na kwamba karatasi ya idhini imepatikana)

Appendix v Summary of Findings in Children below five years

MONTH	Lab No	Age (Months)	Elisa O.D	PAGE	G-type	P-TYPE	Correlation	Untypeable	Mixed infection
Aug 2012	S3	48	0.630	long	G9	P8	G9P[8]		
	S7	5	1.474	long	G1	P8	G1[P8]		
	S12	9	1.645	long	G1	P8	G1[P8]		
Sep 2012	S15	14	1.965	short	G3	P8	G1P[8]		
	S19	58	1.231	long	G9	P8	G9P[8]		
	S23	23	1.383	long	G1	P8	G1P[8]		
Oct 2012	S27	18	1.312	long	G1	P6/P8			P6/P8
Nov 2012	S30	24	1.254	long	G9	P[8]	G9[P8]		
	S34	3	1.357	long	G9	P8	G9[P8]		
	S37	28	0.860	long	G1	P8	G1[P8]		
	S42	8	0.770	long	G1	P8	G1P[8]		
	S45	5	1.461	long	G1	P8	G1P[8]		

Dec 2012	S50	23	1.598	long	G9	P8	G9P[8]		
	S54	11	1.239	long	G1	P8/P6			P8/P6
Jan 2013	S60	7	1.464	short	G9	P6	G9[P6]		
Feb 2013	S71	11	1.676	short	G9	P6	G9P[6]		
	S74	28	1.280	long	U.T	U.T		P andG U.T	
Mar 2013	S81	8	0.321	short	G3	P8	G3P[8]		
	S85	2	1.781	long	G1	P8	G1[P8]		
	S90	7	1.625	long	G1	P8	G1P[8]		
Apr 2013	S94	18	1.394	short	G3	P8	G3P[8]		
	S98	21	1.354	short	G3	P8	G3P[8]		
	S101	35	1.421	long	G9	P8	G9P[8]		
	S103	10	0.521	long	G1	P8	G1P[8]		
	S105	4	0.871	long	G1	P8	G1P[8]		

May 2013	S110	11	0.821	long	G1/G9	U.T		P. UT	G1/G9
Jun 2013	S117	14	1.426	long	G9	P8	G9[P8]		
	S121	8	1.345	long	G1	P[8	G1P[8]		
Jul 2013	S126	8	1.472	long	G1	P[8]	G1P[8]		
	S128	45	1.383		U.T	U.T		PG U.T	

P. UT- P-type untypeable

PG U.T- both P-type and G-type untypeable

Appendix vi Summary of Findings in HIV Adults

Month	**Age (yrs)**	**Lab N.O**	**Sex**	**ELISA O.D**	**PAGE**	**G-Type**	**P-type**	**Correlation**	**Mixed infection**	**Untypeable**
Aug 2012	21	S9	M	1.342	long	G9	P[8]	G9P[8]	0	0
Nov	56	S35	M	1.562	short	G9	P[6]	G9P[6]	0	0

2012										
	24	S47	F	0.321	short	G3	P[8]	G3P[8]		
	54	S52	F	1.423	long	G9	P[8]	G9P[8]		
Jan	61	S62	F	0.456	long	G9	P[8]	G9P[8]	0	0
2013										
	63	S93	F	0.724	long	G9	P[8]	G9P[8]		
March	43	S103	F	1.247	long	G9	P[8]	G9P[8]	0	0
	36	S109	F	0.837	short	G9	P[6]	G9P[6]		
	41	S114	M	0.495	short	G3	P[6]	G3P[6]		
Apr	53	S126	F	0.256	long	G9	P[8]	G9P[8]	0	0

Appendix vii Ethical Clearance

This study was approved byEthical Review Committee (ERC) of Kenya Medical Research Institute (KEMRI) on 31/07/2012. It was voluntary and parents or guardians signed a concept document to allow participation of their children in the study. Personal identification information was removed from the faecal samples for viral gastroenteritis analysis, and the samples were assigned new laboratory numbers. This study did not involve any invasive procedure. The patient collected the stool in the containers in the normal defecation process. This study therefore did not subject the patient/ subject to any pain.

Subjects had direct benefit since they were tested for rotavirus in the clinic using rotavirus rapid kit. Also the results of the study has been shared with KEMRI and other relevant authorities, who will take measures to advice the general public, based on the results, and take the necessary course(s) of action. The results obtained also have further provided information on circulating strains in the region.

Appendix viii SCC

Appendix ix Publication